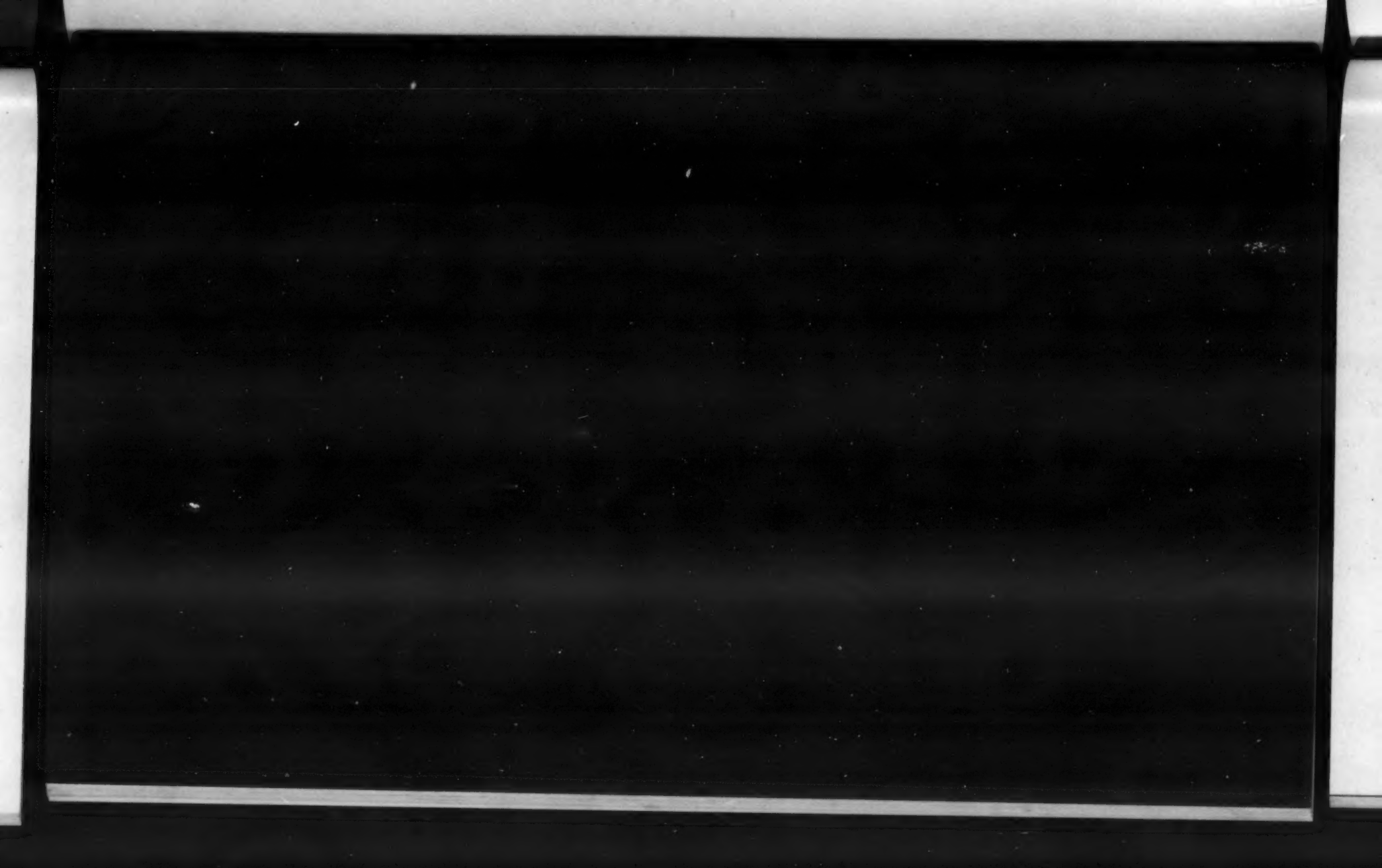


ARCHIVES OF PATHOLOGY

VOLUME 43 JUNE 1947 NUMBER 6

INTRANUCLEAR INCLUSION BODIES IN THE KIDNEYS OF WILD RATS

JEROME T. SYVERTON, M.D.
ROCHESTER, N. Y.
AND
CARL L. LARSON, M.D.*
BETHESDA, MD.

INTRANUCLEAR inclusion bodies have been noted in the renal cells of man and in those of a wide variety of mammals and birds.[1] Although cellular inclusions are accepted as evidence suggestive of the activity of a virus, efforts to recover a causative agent from inclusion-bearing renal tissues have to the present been unsuccessful. Accordingly, when we found renal tubular cells of wild rats captured in Rochester, N. Y., to contain intranuclear inclusion bodies of Cowdry's[2] type B, experiments were designed with a view to learning (*a*) the incidence of the occurrence of the intranuclear inclusion bodies in wild rats and the intensity of involvement of the renal tubular cells, (*b*) whether the causal agent of the intranuclear inclusion bodies is of an infectious nature and (*c*) whether similar intranuclear changes could be produced by employing a foreign substance such as was used by Olitsky and Harford[3] to induce the formation of inclusion bodies. This paper reports the results of our studies.

Intranuclear inclusion bodies were first recorded as being present in the renal cells of rats by Hindle and Stevenson.[4] Hindle[5] in further studies observed intranuclear inclusion bodies to be present almost invariably in the kidneys of London sewer rats, whereas they were absent from

* Fellow in the Medical Sciences of the National Research Council.

From the Department of Bacteriology, University of Rochester School of Medicine and Dentistry.

A preliminary report was published in the Proceedings of the Society of American Bacteriologists (J. Bact. **45**:80, 1943).

1. (*a*) For related bibliography, see Findlay, G. M., in Doerr, R., and Hallauer, C.: Handbuch der Virusforschung, Berlin, A. Hirschwald, 1938, pp. 337-339. (*b*) Kinney, T. D.: Am. J. Path. **18**:799, 1942.

2. Cowdry, E. V.: Arch. Path. **18**:527, 1934.

3. Olitsky, P. K., and Harford, C. G.: Am. J. Path. **13**:729, 1937.

4. Hindle, E., and Stevenson, A. C.: Tr. Roy. Soc. Trop. Med. & Hyg. **23**:327, 1929-1930.

5. Hindle, E.: Nature, London **129**:796, 1932.

country rats. Hindle and Coutelen,[6] in Paris, and Rector,[7] in St. Louis, found similar inclusions in the kidneys of wild rats. Cowdry [8] and Cowdry, Lucas and Fox [8] concluded from morphologic studies of sections provided by Hindle [9] that these inclusions belong to type B. They stated further concerning these bodies, "we entertain the possibility of virus action and await experimental proof." Rector [7] attempted experimentally to produce similar inclusion bodies in 8 normal white rats by injecting a Berkefeld-N filtrate prepared from inclusion-containing tissue derived from 2 wild rats. However, when these 8 white rats were killed at intervals up to twenty days after injection and their kidneys examined microscopically, no inclusion bodies were found.

MATERIALS AND METHODS

Rats.—One hundred and thirty-nine wild rats (Rattus norvegicus) were utilized to provide kidneys for histologic study and for tissue transfer. The source of these rats was as follows: Rochester provided 117 rats, of which 65 were caught in or near the animal house of the medical school, to which a seasonal immigration from outlying districts occurs, and 52 were captured in parts of the city where they had ready access to sewers and refuse; 5 came from farms in upstate New York; 12 were captured on the water front in San Francisco; 5 were sent from Denver.

Sixty-two normal albino rats (Rattus norvegicus) of the Wistar strain were used. Of these rats, 33 were employed as recipients for suspensions of renal tissues known to contain many inclusion bodies, and 29 served as hosts for injections of aluminum hydroxide gel.

Preparation of Tissues for Microscopic Examination.—Immediately after a rat was killed, its kidneys were removed under aseptic precautions and hemisectioned longitudinally. One half of each kidney was prepared for microscopic study either by fixation in Zenker's (5 per cent acetic acid) fluid for subsequent staining with hematoxylin and eosin and by Giemsa's method or by employing the frozen section technic and staining the sections with hematoxylin and eosin. The other half of each kidney was used for transmission to animals and for bacteriologic studies.

Preparation of Suspensions for Infection.—Suspensions of the renal tissues of wild rats were prepared for injection into albino rats according to the following technic. Kidneys immediately after their removal from each wild rat were made ready for microscopic examination by employing the frozen section technic and staining the sections with hematoxylin and eosin. Only tissues found on microscopic examination to contain numerous inclusion bodies (4 plus) [10] were accepted

6. Hindle, E., and Coutelen, F.: Compt. rend. Soc. de biol. **110**:870, 1932.

7. Rector, L. E.: Proc. Soc. Exper. Biol. & Med. **34**:700, 1936.

8. Cowdry, E. V.; Lucas, A. M., and Fox, H.: Am. J. Path. **11**:237, 1935.

9. These histologic sections were prepared from tissues removed from rats in England and sent by Dr. E. Hindle to Dr. E. V. Cowdry (Am. J. Path. **11**:237, 1935).

10. The stained sections were systematically examined, mechanical stage, a 10 × ocular, and 4 mm. and 1.8 mm. objectives being employed. The intensity

as satisfactory for passage. Such tissues were triturated with alundum in Locke's solution to yield a 10 per cent suspension. Meat extract broth was substituted for Locke's solution when filtration experiments were contemplated. These suspensions were centrifuged horizontally at 1,500 revolutions per minute for thirty minutes, and each supernatant fluid directly, or after filtration, was used undiluted in the inoculation of albino rats.

Alumina gel type C was made according to the method of Willstätter and Kraut [11] as modified by Sabin.[12]

Preparation of the Albino Rats Used as Recipients for Inclusion-Containing Tissue.—The possibility that naturally acquired inclusion bodies might be present in the renal cells of the white rats employed as recipients for test materials was considered. Accordingly, the left kidney from each of 33 white rats was removed under aseptic precautions [13] through a curved incision that paralleled the twelfth rib in the left lumbar region. Tissue sections from each kidney were prepared and studied microscopically. Inclusion bodies were not found in a single kidney. Accordingly, when all of these albino rats had made an uneventful recovery from nephrectomy ten to twenty days later, they were inoculated with a suspension of inclusion-containing tissue.

DESCRIPTION OF THE NATURALLY OCCURRING DISEASE

Our description of the naturally occurring disease [14] is necessarily inadequate because it was impossible to keep wild rats under observation for prolonged periods.

Observations on the naturally occurring disease were limited to inspection of a single group of 10 rats during a three week period and of the remaining 129 rats immediately following capture.

The wild rats whose kidneys contained many inclusion bodies at the time of their capture were indistinguishable on gross inspection from rats whose kidneys contained no inclusion bodies. It was noted, however, that inclusions were absent from immature rats that were less than half grown and from rats captured on farms. On the other hand, battle-scarred males, obviously old, had kidneys that contained the greatest number of inclusion bodies. It is noteworthy that most of the old rats also yielded on cultures of tissues Leptospira icterohaemorrhagiae [15] and Salmonella typhimurium. Only rarely were rats found that were not well nourished and in excellent physical condition. It was impossible in

---

of involvement as shown by the number of cells containing intranuclear inclusion bodies in each high power field was designated as follows: 0 = no inclusion bodies, 1+ = 0 to 1 inclusion body, 2+ = 2 to 6 inclusion bodies, 3+ = 6 to 10 inclusion bodies, and 4+ = 10 or more inclusion bodies.

11. Willstätter, R., and Kraut, H.: Ber. ü. d. chem. Gesellsch. **56**:149, 1923.

12. Sabin, A. B.: J. Exper. Med. **56**:307, 1932.

13. Ether anesthesia was employed for all operative procedures.

14. In the present paper the term "disease" is used to designate a morbid condition that is manifested by characteristic intracellular alterations in the renal tubular cells.

15. Syverton, J. T.; Stiles, W. W., and Berry, G. P.: J. Bact. **36**:285, 1938.

the latter group to relate their poor physical condition to the bacteriologic and histologic observations.

These observations led us to believe that if the inclusion bodies were associated with an infection, the infection was inapparent. The association in many animals of inclusion disease and either leptospiral infection or salmonellosis, or both, suggested that these two diseases also were present as inapparent infections in hosts that served as carriers.

PATHOLOGIC AND HISTOLOGIC EXAMINATIONS

Since it was apparent early in the present studies that there were no external signs diagnostic of the disease, it was the histopathologic examination of renal tissue that determined when a diagnosis of renal inclusion

TABLE 1.—*Incidence of Renal Inclusion Disease in Two Hundred and One Rats (Rattus norvegicus)*

Type of Rat	Rats Whose Renal Cells Contained Inclusion Bodies		Rats Whose Renal Cells Did Not Contain Inclusion Bodies	
	Number	Per Cent	Number	Per Cent
Wild	68	49	71	51
Albino	0	0	62	100

disease was made. Accordingly, the kidneys from each of 139 wild rats and 62 stock albino rats of the Wistar strain were examined macroscopically and microscopically for pathologic alterations.

The kidneys from wild rats showed wide variation in their external appearance. Young wild rats and stock albino rats yielded kidneys from which the capsules separated readily, leaving uniformly smooth cortical surfaces. In contrast

TABLE 2.—*Intensity of Involvement in Sixty-Eight Wild Rats Proved to Have Renal Inclusion Disease*

Involvement Graded According to the Approximate Number of Inclusion Bodies per High Power Field	Rats	
	Number	Per Cent
1+ (from none to 1)	22	32
2+ (from 2 to 6)	17	25
3+ (from 6 to 10)	14	20
4+ (10 or more)	15	22

to these findings, old wild rats almost without exception had kidneys that presented cortical surfaces with extensive focal scarring following removal of the adherent capsules. The scars were few for the most part, irregularly distributed to involve all parts of the cortex, variable in size and pyramidal in shape. On longitudinal hemisection the scars were found to extend into the medullary substance.

One or more sections from each kidney were examined microscopically to determine whether intranuclear inclusion bodies were present and, if so, the relative number, the location and the character of any

attendant lesions. The results of the microscopic examination of renal tissue from each rat are summarized in tables 1 and 2.

It can be seen that intranuclear inclusion bodies were present in renal tissue from 68 (49 per cent) of the 139 wild rats studied. The numbers of inclusion bodies were approximated by utilizing an arbitrary scale ranging from 1 plus to 4 plus.[10] As may be noted in table 2, this rough determination of the intensity of occurrence of inclusion bodies resulted in the finding that there was about the same number of hosts for each grade. The absolute numbers of inclusions ranged from 1 to 75 per high power field.

In a single microscopic field as many as 40 per cent of the epithelial cells lining the convoluted tubules contained the inclusion bodies. On the other hand, the inclusion bodies were not found in the epithelial cells either of the collecting tubules or of the capsules of Bowman. In most instances the inclusion occurred as a single, homogeneous, smooth-appearing, pinkish purple-staining body situated within, and largely replacing, the nucleoplasm (fig. 1). More usually, the basophilic chromatin was concentrated around the inclusion body, and the relatively small basophilic nucleolus had migrated to the nuclear membrane (fig. 2). In some cells, however, an areola surrounded the inclusion body, which exhibited marked acidophilic properties as shown by an intense scarlet-red coloration, and its presence was associated with margination of the basophilic chromatin network and nucleolus (fig. 3). Inclusion-bearing cells frequently were greatly hypertrophied, being several times normal size (figs. 4 to 6). These host cells stood out in contrast to normal cells and to other cells of normal size that contained inclusion bodies.

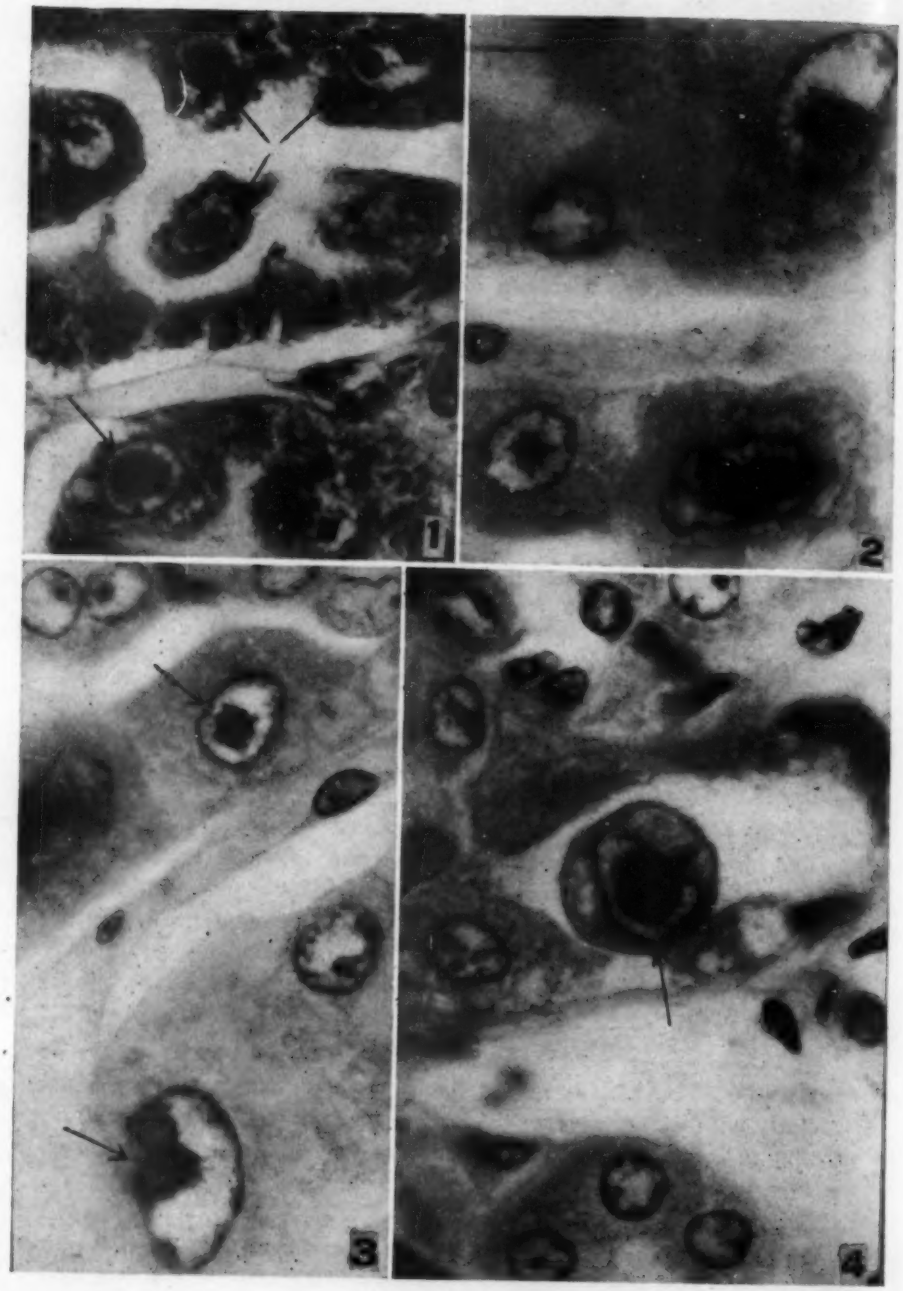
The concomitant renal lesions were variable. Grossly visible depressions and scars were found to correspond to areas of chronic pyelonephritis as shown by cellular infiltration, varying amounts of fibrosis and damage of glomeruli and tubules. The chronic inflammatory cells were predominantly lymphocytes but included many plasma cells and monocytes. (A representative section is pictured in figure 7.) These findings were without apparent relation to the presence of intranuclear inclusion bodies.

The histologic examination of tissues from 139 wild rats and from 62 laboratory albino rats made it apparent that the only distinctively diagnostic finding in renal inclusion disease was the presence in normal to greatly enlarged renal epithelial cells of a homogeneous, hyalin-like, pinkish purple circular body replacing the nucleoplasm and basophilic chromatin network.

BACTERIOLOGIC STUDIES

Because of the possibility that inclusion bodies might be associated regularly with a cultivable agent, bacteriologic studies were made in an attempt to isolate from renal tissue known to contain inclusion bodies, bacteria, fungi and pleuropneumonia-like organisms.

A triturated suspension of kidney tissue from each of the first 12 rats studied was cultured by inoculating Douglas' broth, rabbit's blood–agar plates, eosin–methylene blue agar plates, Fletcher's mediums, Löffler's mediums and deep meat tubes for incubation under aerobic and anaerobic conditions. Bacteriologic studies of most of the remaining 127 rats were limited to the inoculation of rabbit's blood–agar plates to detect the presence of salmonellas or contaminants and to the



Figures 1, 2, 3 and 4

(See legend on opposite page)

inoculation of Fletcher's mediums or sterile tap water to detect the presence of L. icterohaemorrhagiae.

The bacteriologic studies showed that extraneous contaminants were present rarely, S. typhimurium irregularly and L. icterohaemorrhagiae in 68 of the 139 rats (49 per cent) whose kidneys were investigated.

The results of the bacteriologic studies yielded no evidence to indicate that the presence of the inclusion bodies in the renal tissues was regularly associated with a single species of cultivable agent. It is of interest that renal inclusion bodies and L. icterohaemorrhagiae were demonstrated in the same percentage of rats (49 per cent). When attempts were made to relate these results for single animals, it became apparent that this finding represented a fortuitous coincidence, since some animals yielded leptospiras only, others inclusion bodies only, others both, and others neither. It was noted, however, that obviously old and senile rats not infrequently carried L. icterohaemorrhagiae, S. typhimurium and numerous inclusion bodies.

ATTEMPTS TO INDUCE RENAL INCLUSION DISEASE WITH TISSUE SUSPENSIONS

If a virus is the causative agent of renal inclusion disease of rats, tissue from kidneys known to contain numerous inclusion bodies might on transfer to susceptible hosts yield identical intranuclear alterations. In experiments designed to demonstrate a virus as the etiologic agent, suspensions of renal tissues from wild rats known to have the disease were injected into 33 albino rats.

The suspensions were prepared, as described, from the kidneys of wild rats shortly after removal. The kidneys utilized were limited to those which were known from microscopic examination of sections prepared by the frozen tissue technic to contain numerous inclusion bodies. The 33 albino rats received by the subcutaneous or the intraperitoneal route 1 cc. of a 10 per cent suspension; 24 animals were given unfiltered tissue suspension, and 9 received Berkefeld-V filtrate. These animals were observed for from three to eighty-four days after injection.

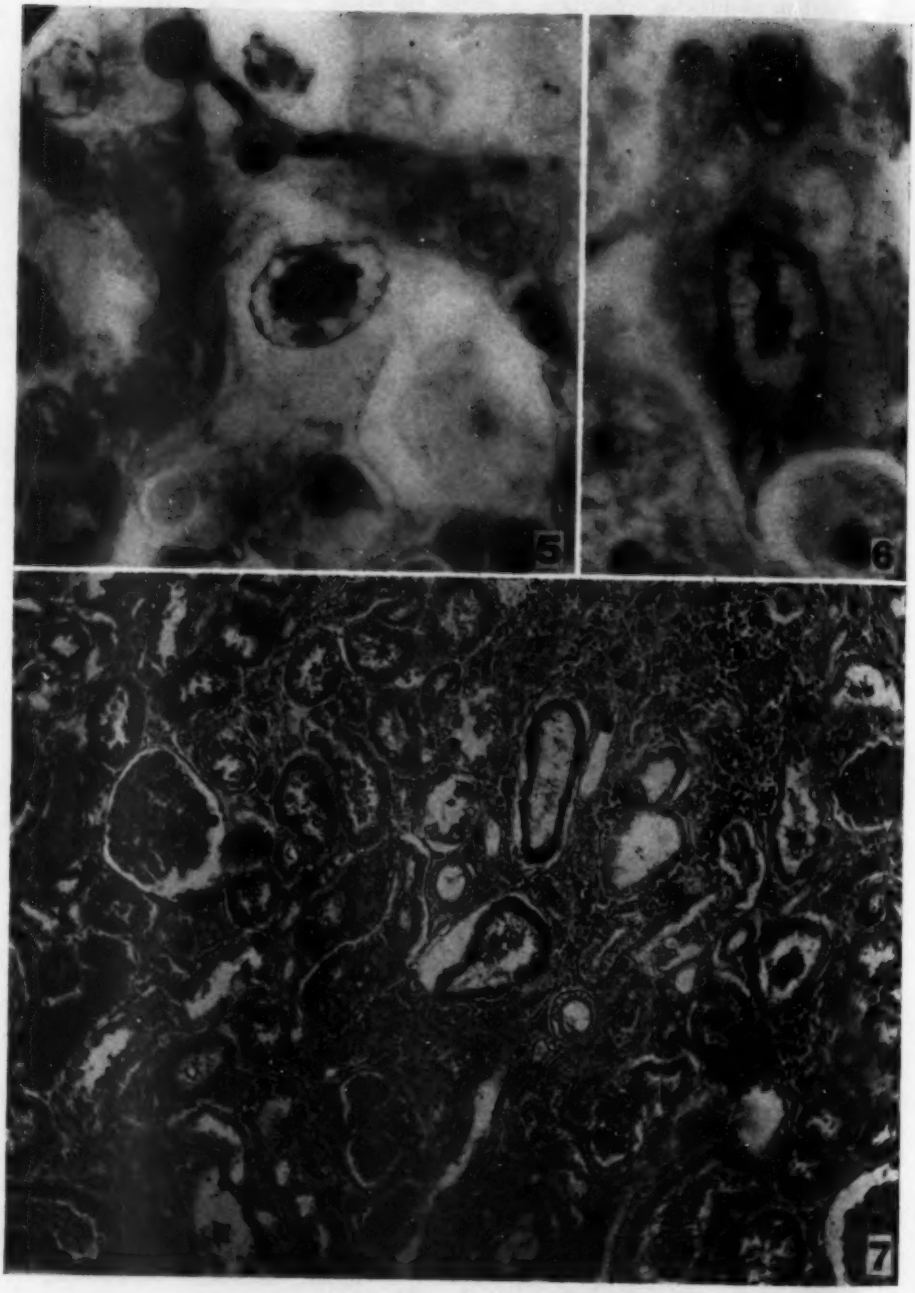
EXPLANATION OF FIGURES 1 TO 4

Fig. 1.—Section of kidney from wild rat 66. Each of 4 epithelial cells contains a single intranuclear inclusion body (arrows). Hematoxylin and eosin; × 1,500.

Fig. 2.—Section of kidney from wild rat 187. Each of two greatly enlarged cells contains an intranuclear inclusion body. It can be seen that the basophilic chromatin is concentrated around the inclusion bodies and that the nucleoli are situated peripherally. Hematoxylin and eosin; × 1,500.

Fig. 3.—Section of kidney from wild rat 175. The intranuclear inclusion bodies present in two cells (arrows) are markedly acidophilic and somewhat granular, resembling a type A intranuclear inclusion body.

Fig. 4.—Section of kidney from wild rat 82. A single, greatly enlarged cell and nucleus with its contained inclusion body (arrow) stands out in contrast to the normal cell. Hematoxylin and eosin; × 1,500.



Figures 5, 6 and 7

(See legend on opposite page)

When none of the recipients showed evidence of clinical disease during the period of observation, and when microscopic examination of renal and salivary gland tissues from each rat failed to reveal intranuclear inclusion bodies, we concluded that a virus probably was not the causative agent of renal inclusion disease of wild rats.

ATTEMPTS TO PRODUCE INCLUSION BODIES BY INJECTING A CHEMICAL SUBSTANCE

When transmission experiments utilizing tissues known to contain inclusion bodies failed to yield clinical or histologic evidence of renal inclusion disease, resort was made to the experimental approach successfully employed by Olitsky and Harford [8] for the induction of intranuclear inclusion bodies by injection of chemical substances. These investigators demonstrated intranuclear inclusion bodies in the phagocytic mononuclear and giant cell constituents of foreign body tissue reactions that followed subcutaneous injection of selected aluminum and ferric compounds and carbon. Since we had found the renal inclusion disease of rats to be largely confined to animals captured near sewers and dumps, it seemed possible that a toxic agent, e. g., a metal, might be ingested and absorbed from the gastrointestinal tract, to produce changes in renal epithelial cells during excretion. Accordingly, we selected aluminum hydroxide to be injected intravenously in animals believed to be susceptible.

Twenty-nine mature albino rats were selected as recipients. For control purposes, so as to rule out the possibility of preexisting renal inclusion bodies, a kidney was removed from each rat. Forty-five days later, after microscopic study had failed to reveal inclusion bodies in a single one of the kidneys from these normal animals, 0.2 to 0.5 cc. of aluminum hydroxide prepared according to the directions for making Willstätter's type C gel [16] was injected into the tail vein of each of these rats. Fourteen animals died within seventy-two hours after receiving the injection. The data that relate to the 15 survivors are presented in table 3.

It can be seen in table 3 that 14 of the 15 survivors were given a second injection, but that 8 of these died within twenty-four hours. This

16. Willstätter and Kraut.[11] Sabin.[12]

EXPLANATION OF FIGURES 5 TO 7

Figs. 5 and 6.—Sections of kidney from wild rats 175 and 182. Both photographs show additional examples of the cellular alterations that occur in renal inclusion disease of wild rats. It can be seen that two of the cells are enlarged. Hematoxylin and eosin; × 1,500.

Fig. 7.—This section from wild rat 66 is selected to show the type of histologic alteration present in many wild rat kidneys. Damaged glomeruli and tubules, round cell infiltration and fibrotic changes are present. Hematoxylin and eosin: × 150.

made sections available for microscopic study from the remaining kidney and other tissues of 8 animals that survived a single injection for from thirteen to forty-nine days and from 7 that survived two injections whose deaths occurred from thirty-six to ninety-four days after the first injection, or from seven to eighty days after the second injection. It would seem that ample time had been allowed for tissue changes to develop. The results, however, were negative, for microscopic examination uniformly failed to show inclusion bodies.

COMMENT

The discovery of intranuclear inclusion bodies of Cowdry's type B in the renal cells of a rat captured in Rochester, N. Y., led us to sample the rat population in Rochester and other cities of the United States to learn whether this type of change was common among rats. Accordingly, 139 wild rats were procured from Rochester and such widely separated cities as Detroit, Denver and San Francisco. Inclusion bodies were found in the epithelial cells of 49 per cent. The incidence was apparently

TABLE 3.—*Data That Relate to the Attempt to Induce Intranuclear Inclusion Bodies in the Renal Epithelial Cells of Albino Rats by One or Two Intravenous Injections of Aluminum Hydroxide*

Rat	Aluminum Hydroxide Injected Intravenously, Cc.		Time Interval	
	Dose 1	Dose 2	Days to Dose 2	Days to Death
A 1	0.5	0.3	13	13
A 2	0.5	0.2	18	18
A 3	0.2	...	0	23
A 4	0.2	0.3	27	27
A 5	0.25	0.3	27	27
A 6	0.3	0.5	27	27
A 7	0.5	0.3	27	27
A 8	0.5	0.25	27	36
A 9	0.4	0.1	49	59
A 10	0.5	0.4	49	49
A 11	0.5	0.4	49	49
A 12	0.2	0.3	21	70
A 13	0.25	0.3	27	76
A 14	0.5	0.3	14	94
A 15	0.3	0.3	14	94

unrelated to geographic origin, but was highest among old urban rats. On the other hand, inclusion bodies were not found in renal tissues from immature rats, from rats captured in rural areas, or from albino rats, irrespective of their age.

This demonstration of intranuclear inclusion bodies in about half of the wild rats led us to suspect that we might be dealing with a disease caused by a virus, for the presence of inclusion bodies in cells is often

evidence of virus activity. For one type of intranuclear inclusion body (Cowdry's type A) this association is so regular that the microscopic demonstration of type A intranuclear inclusion bodies in tissue cells is commonly accepted as pathognomonic of virus infection. On the other hand, the presence of intranuclear inclusion bodies of Cowdry's type B is but suggestive of virus activity. As the intranuclear inclusion body in renal inclusion disease of rats is of the latter variety, it seemed pertinent that our studies should be directed toward learning whether this malady could be explained by recovery of a virus; if not, toward securing data on which to appraise the significance of the inclusion bodies. Accordingly, our experiments were designed to enable us (1) to isolate a virus and (2) to reproduce the intranuclear alteration by inoculating the inclusion-laden tissues into normal animals and by utilizing aluminum hydroxide, a chemical known to produce inclusion bodies.

Our experiments yielded no evidence that a virus is the etiologic agent of the renal inclusion disease of rats or that the cellular alterations of this disease can be reproduced by the injection of aluminum hydroxide. We can, therefore, but conjecture as to the nature and the mode of action of the agent responsible for the pathologic picture. It was noted that the disease was limited to urban rats and that the incidence of occurrence and the number of inclusion bodies increased with age. Moreover, rats captured in refuse dumps or downtown showed more evidence of the disease than suburban rats. This suggests that repeated ingestion of some toxic agent, perhaps a heavy metal, might be responsible. Another possibility is that the intranuclear inclusion bodies reflect the residuum of an inapparent virus infection of slight pathogenicity and long duration. The demonstration in this same group of rats of inapparent infections with L. icterohaemorrhagiae and S. typhimurium and the capacity of rats to harbor bacteria,[17] rickettsias [18] and viruses [19] as inapparent infections possibly lend some support to this idea.

It is of interest that renal inclusion bodies and recovery of L. icterohaemorrhagiae were demonstrated for the same percentage of rats (49 per cent). However, attempts to relate these findings for single animals made it apparent that this observation represented a fortuitous coincidence, since some animals yielded leptospiras only, others inclusion bodies only, others both, and others neither leptospiras nor inclusion bodies. It was noted, however, that obviously old and senile rats not only showed evidence for extensive inclusion body disease but also carried L. icterohaemorrhagiae and S. typhimurium.

17. Hülphers, G., and Henricson, T.: Svensk vet. tidskr. **48**:197 and 245, 1943; abstracted, Biol. Abstr. **20**:383, 1946.

18. Dyer, R. E.: Am. J. Trop. Med. **21**:163, 1941.

19. Burnet, F. M.: J. Path. & Bact. **42**:213, 1936.

SUMMARY

The kidneys from 139 wild rats (*Rattus norvegicus*) captured in widely separated parts of the United States were examined microscopically for the presence of intranuclear inclusion bodies. When it was found that Cowdry's type B intranuclear inclusion bodies were present in the epithelial cells of the renal tubules of 68 rats (49 per cent), two groups of experiments were carried out to learn whether the causal agent was infectious in nature or a chemical.

Albino rats of the Wistar strain were used as experimental animals. To rule out preexisting inclusion bodies in the kidneys, one kidney from each albino rat was removed for microscopic examination forty-five days before use of the rat.

The first group of experiments employed fresh renal tissues that were known from examination of sections prepared by the frozen section technic to contain numerous inclusion bodies. Filtered and unfiltered suspensions of these tissues were injected into 33 normal albino rats by subcutaneous and intraperitoneal routes. When animals were killed in from three to eighty-four days after injection, none showed any inclusion bodies.

In the second series of experiments, Willstätter's type C alumina gel (0.2 to 0.5 cc. in one or two doses) was injected intravenously into 29 rats. Of these, 15 survived and died or were killed in from thirteen to ninety-four days after injection. Again, in none of the rats were intranuclear inclusion bodies found.

The photographs were made by Mr. Mervyn C. Orser.

PULMONARY ADENOMATOSIS RESEMBLING JAGZIEKTE IN THE GUINEA PIG

ROBERT F. NORRIS, M.D.
PHILADELPHIA

PULMONARY adenomatous lesions of the guinea pig were described by Sternberg [1] in 1904 and Spronck [2] in 1907. In 1926 Grumbach [3] induced adenomatous lesions in the lungs of guinea pigs by inoculating a diphtheroid bacillus isolated from the lymph nodes of a patient with Hodgkin's disease. Grumbach was impressed by the fact that these lesions were similar to those of jagziekte of sheep. Cowdry and Marsh,[4] who also examined the slides, agreed that the experimentally induced lesions resembled those of jagziekte as well as those of the progressive pneumonia occurring in the sheep of Montana.

The present report is concerned with a guinea pig which had adenomatous lesions of one lung, also resembling jagziekte of sheep. Although the guinea pig was inoculated prior to death with pleural fluid from a patient suspected of having pulmonary tuberculosis, it will be seen from the following clinical history that the inoculation probably was not responsible for the lesions.

REPORT OF CASE

W. R. B., a male Negro aged 18, was admitted to the Hospital of the University of Pennsylvania, May 7, 1946, complaining of pain in the left side of the chest. Following the onset of a cold several days before admission, he felt feverish and began to cough up reddish sputum. On admission the diagnosis was lobar pneumonia of the lower lobe of the left lung, which was confirmed by a roentgenogram of the chest. The clinical signs and symptoms disappeared promptly after the patient was treated with sulfadiazine and penicillin, and he was discharged from the hospital on May 15, eight days after admission.

He was readmitted to the hospital on May 24 because of a recurrence of pain in the left side of the chest. At this time a large collection of fluid was present in the left pleural cavity. He was treated by repeated aspirations. Before the pleural effusion had entirely disappeared, he signed his release from

---

From the William Pepper Laboratory of Clinical Medicine, University of Pennsylvania.

1. Sternberg: Verhandl, d. deutsch. path. Gesellsch. **6**:134, 1904.
2. Spronck, C. H. H.: Nederl. tijdschr. v. geneesk. **43**:1033, 1907.
3. Grumbach, A.: Bull. Assoc. franç. p. l'étude du cancer **15**:213, 1926.
4. Cowdry, E. V., and Marsh, H.: J. Exper. Med. **45**:571, 1927.

the hospital on June 8 and was not seen again in the dispensary until Jan. 4, 1947, when he was suffering from acute gonorrheal urethritis. At this time in a roentgenologic examination of the chest the heart and lungs were normal, but the left side of the diaphragm fluoroscopically was slightly flattened and the left costophrenic sulcus was blunt. Those abnormalities were the only sequelae of the previous pneumonia and pleural effusion.

During the second admission, several specimens of aspirated pleural fluid were uniformly clear and straw-colored and were sterile when cultured by routine bacteriologic methods. One specimen cultured for tubercle bacilli likewise showed no growth. Two guinea pigs were inoculated with this specimen. Both animals

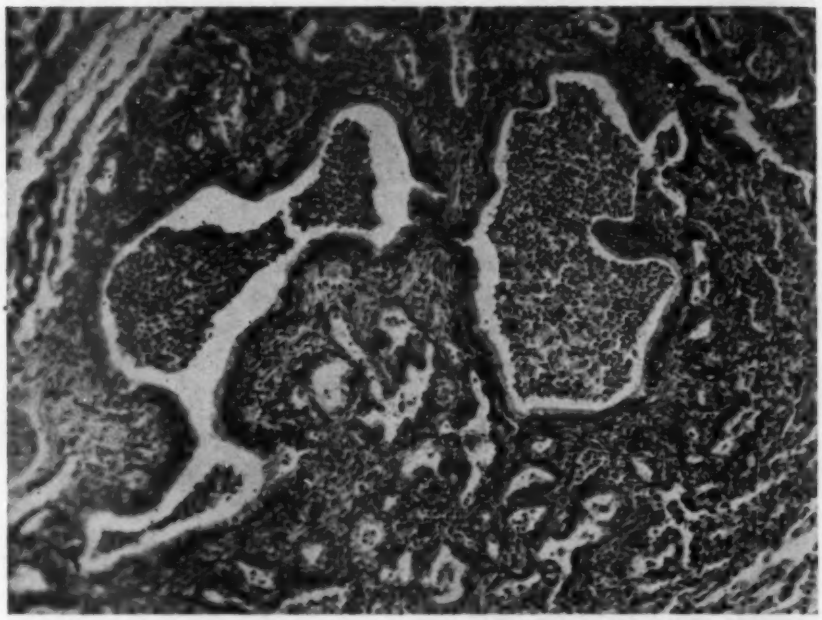


Fig. 1.—There are papillary projections of columnar epithelium in two widely dilated small bronchi. The lumens contain many polymorphonuclear neutrophils and large mononuclear and polymorphonuclear phagocytes. The neighboring alveoli are lined by epithelial cells of like character. Adenomatous proliferation of the epithelium is extending centrifugally, and the uninvolved alveoli appear compressed.

were alive and appeared well when killed two months after inoculation. The organs of one guinea pig were grossly and microscopically normal. The lesions found in the other guinea pig will be described in detail.

Necropsy of the Guinea Pig.—The technician who killed the animal did not observe any gross lesions at the necropsy. It was not until the microscopic sections were prepared that the following abnormalities were discovered.

In one lung there were discrete and confluent foci of adenomatous epithelial proliferation. In roughly circular zones about the bronchi the neighboring alveoli

were completely lined by epithelial cells of the same character. An adenomatous appearance thus resulted. Peripherally the lesions were not encapsulated, but the nearby alveolar stroma was condensed. The epithelial proliferation was sharply marginated, but throughout the circumference of the lesions epithelial cells were proliferating into previously normal alveoli but had not yet completely lined them. The parenchyma of the lung between lesions showed no abnormalities other than atelectasis, which was possibly due in part to compression (fig. 1).

The epithelial cells comprising the lesions were identical in both the bronchi and the alveoli. They were larger and more swollen in appearance than those of normal bronchi, and none were ciliated. Although in most areas the bronchi and alveoli were lined by a single layer of epithelial cells, in places the cells were heaped up to form papillary folds which projected into the lumens. Most of these papillae had cores of fibrous tissue. Individually the cells were generally of uniform size and showed little pleomorphism. The nuclei were usually oval, vesicular and moderately hyperchromatic. Small basophilic nucleoli were often present. Mitoses were not seen. Most often the nuclei were basilar. The cytoplasm was slightly granular but translucent and practically colorless (fig. 2*A*).

Within the cytoplasm of many cells were faintly stained oval bodies, approximating the size of nuclei, but often larger, which were more opaque than the surrounding cytoplasm and which appeared to contain minute darkly staining granules. The individual granules were surrounded by narrow translucent zones. Stained with Giemsa or phloxine and methylene blue, however, these granules were less distinct than with hematoxylin and eosin. The bodies containing these granules were sharply marginated, but distinct enclosing membranes were not distinguished. Most of these bodies were situated between the nucleus and the free margin of the cell. At times they were free in the lumens. Some of these were engulfed by large mononuclear or polymorphonuclear cells resembling phagocytes (fig. 2*B*).

Within the lesions the alveolar septums were thickened by increased amounts of collagenous and reticular tissue and by small numbers of extravasated plasma cells, mononuclear phagocytes and polymorphonuclear neutrophils. Only a few large foci of lymphoid hyperplasia were seen in the vicinity of bronchi, and there was no scarring or proliferation of myxomatous fibrous tissue.

Within the lumens of the bronchi were large numbers of polymorphonuclear neutrophils and a few large mononuclear phagocytes. Large mononuclear phagocytes, some of which were multinuclear, were also present in the lumens of the alveoli, but in only a few were polymorphonuclear neutrophils present. Gram and Giemsa stains did not demonstrate bacteria. Unfortunately when the lesions were discovered there was no unfixed tissue from which further bacteriologic studies could be made.

In a section from the opposite lung there were no large adenomatous lesions, but there was reduplication of the epithelium in several of the small bronchi. Extending into the alveoli adjacent to these bronchi were small areas of epithelial proliferation resembling those in the other lung. Inflammatory exudate and lymphoid hyperplasia were not present in this lung. Except for compensatory emphysema, this lung showed no other lesions.

In a section of the spleen, small zones of fibrosis, not yet hyaline, encircled many of the malpighian bodies, but no other lesions were seen. Nothing abnormal was seen in sections of the liver, a kidney and inguinal lymph nodes.

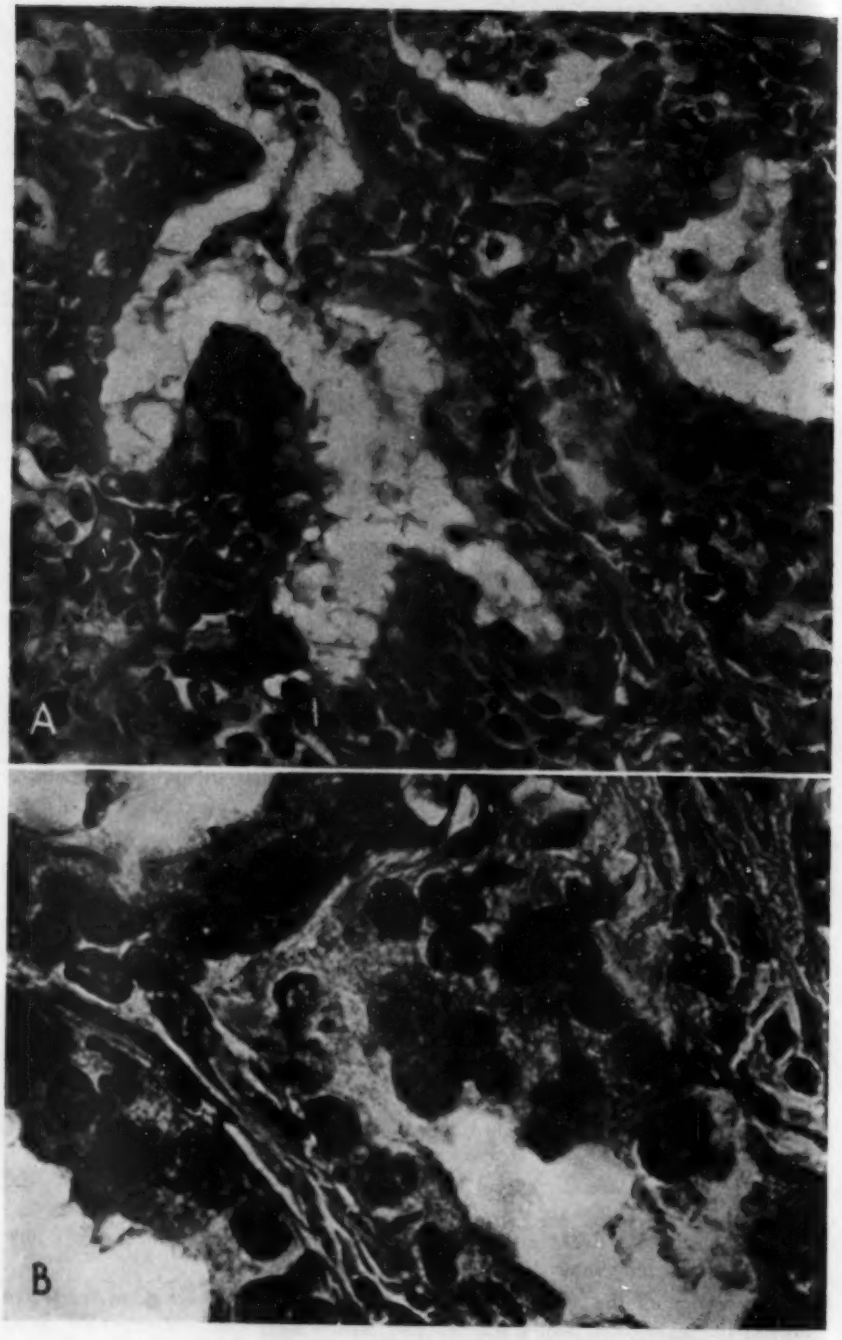


Figure 2

(See legend on opposite page)

COMMENT

The pulmonary adenomatous lesions described are thought to have been essentially benign in that invasion of the alveoli occurred without apparent destruction of the septums. Also the individual epithelial cells were well differentiated and were generally uniform in contour. However, blocks were not available from a sufficient number of organs to exclude the presence of metastasis. Since the epithelium of the smaller bronchi corresponded with that lining the alveoli, it may be assumed that epithelial proliferation began in the bronchi and extended peripherally to involve the neighboring alveoli.

So far as can be determined from the descriptions, the lesions of the present case were similar to those in guinea pigs described by Sternberg [1] and later by Spronck,[2] and to those induced experimentally by Grumbach.[3] Sternberg likewise believed that the epithelial proliferation began in the smaller bronchi.

Since the lesions in the present case also were thought to resemble the jagziekte occurring in sheep, at the suggestion of Dr. Baldwin Lucké slides were submitted to Major W. A. Bennett, of the Army Institute of Pathology, Washington, D. C., and to Dr. C. L. Davis, of the Bureau of Animal Industry, Denver, and they expressed the following opinions. Major Bennett expressed the belief that the lesions were compatible with jagziekte but pointed out that it is unusual for the disease to begin in the smaller bronchi. Dr. Davis, however, expressed doubt as to whether a diagnosis of jagziekte was justified, especially since the disease is primarily one of the ovine species. Both emphasized the likelihood that the epithelial hyperplasia was secondary to chronic inflammation.

In a consideration of the arguments for and against a relationship with jagziekte in the present case, it may be said that the lesions resembled those in sheep in that the epithelial proliferation did not appear to be cancerous and the individual epithelial cells were large nonciliated columnar cells with clear cytoplasm. The papillary projections were also similar to those of jagziekte and the large oval or circular granular bodies seen in the cytoplasm of the cells and in the lumens of the alveoli resembled those observed by Dungal [5] in the sheep of Iceland. The observation that the epithelial proliferation apparently originated in the small bronchi may be considered as one which would differentiate the condition from jagziekte, because Cowdry [6] reported that the lesions

5. Dungal, N.: Proc. Roy. Soc. Med. **31**:497, 1938; Am. J. Path. **22**:737, 1946.
6. Cowdry, E. V.: J. Exper. Med. **42**:323 and 335, 1925.

Fig. 2.—With greater magnification (*A*) papillary projections of translucent columnar alveolar epithelium are apparent. The epithelial cells show little pleomorphism. In many cells, between the nucleus and the free margin are opaque spherical or oval bodies containing darkly staining granules. In *B*, with still greater magnification (oil immersion objective), several large spherical bodies containing darkly staining granules may be seen lying free in the lumen of the alveolus.

of the latter begin in the alveoli and not in the bronchi. Mitchell,[7] de Kock,[8] and Dungal [5] stated, however, that the lesions may originate in the smaller bronchi. There was also absence of marked lymphoid hyperplasia, described by Mitchell [7] as common in jagziekte. But de Kock [8] was unable to confirm this characteristic and raised the question whether Mitchell might have been dealing with two separate diseases. In the present case, the lack of proliferation of myxomatous and fibrous stroma is not necessarily an argument against the diagnosis, since these changes usually occur late in the course of the disease and are not always present.

It is uncertain whether the inflammatory exudate present especially in the bronchi represented antecedent bronchitis which initiated the proliferation of epithelium or secondary infection. The fact that acute inflammatory exudate was more abundant in the bronchi than in the alveoli and that inflammatory exudate was lacking in the opposite lung favors the latter hypothesis, which, if true, is in agreement with the observation [9] that secondarily acquired pneumonia is not uncommon in jagziekte.

From the evidence presented it may be concluded that the adenomatous lesions of the present case are morphologically similar to jagziekte of sheep. Except for this resemblance, there is no evidence that the lesions are etiologically identical.

Although the cause of jagzeikte is still unknown, most observers believe that the disease is infectious.[5] Attempts to transmit the disease from sheep to other animals, including guinea pigs, have not been successful. Nevertheless, the fact that pulmonary adenomatosis occurs in guinea pigs indicates that if jagziekte is ultimately proved to be an infection it may yet be transmitted to guinea pigs under proper conditions of experiment.

Since the patient whose pleural fluid was injected into the guinea pig recovered from the lobar pneumonia and does not at present show any roentgenologic evidence of pulmonary disease, it is unlikely that the inoculation was significant in the causation of the pulmonary adenomatosis of the guinea pig.

SUMMARY

Pulmonary adenomatosis involving one lung of a guinea pig is described. The lesions resemble those of jagziekte occurring in sheep. Although the guinea pig was originally inoculated with the pleural fluid of a patient convalescing from lobar pneumonia, there is no evidence that the patient suffered from a similar disease.

---

7. Mitcheli, D. T., in Third and Fourth Annual Reports of the Director of Veterinary Service, Union of South Africa, 1915, p. 585.

8. de Kock, G., in Fifteenth Annual Report of the Director of Veterinary Service, Union of South Africa, 1929, p. 611.

9. Dungal.[5] de Kock.[8]

STRUCTURAL CHANGES IN THE KIDNEYS OF RATS WITH EXPERIMENTAL CHRONIC HYPERTENSION

BÉLA HALPERT, M.D.
OKLAHOMA CITY
AND
ARTHUR GROLLMAN, Ph.D., M.D.
DALLAS, TEXAS

IN A PREVIOUS communication studies of the structural changes observed in the visceral blood vessels, the heart and the kidneys of rats with experimental chronic hypertension were reported.[1] It was stated that the blood vessels revealed minimal or no microscopic changes and that the left cardiac ventricle showed hypertrophy with its concomitant changes. The latter changes were usually commensurate with the degree and the duration of the observed elevation of blood pressure. Renal lesions apparently responsible for the hypertension were observed in every kidney to which a ligature was applied. Focal changes attributed to the hypertension were observed in the opposite, unligated kidney.

In this paper, studies are reported on an additional series of rats in which experimental chronic hypertension had been induced by various methods. Particular attention is paid to the renal lesions producing the hypertension and to the renal lesions resulting from the hypertension.

METHOD

Twenty piebald rats of the Evans-McCollum strain were used. In 13 of these a figure of eight ligature was applied to the right kidney as previously described, the left kidney being left intact.[2] Within ten to sixteen weeks chronic hypertension developed in 9 of these rats. Of the remaining 4 rats, in which the procedure did not result in hypertension, 3 had a ligature applied then to the left kidney, and in 1 the left kidney was removed. In 2 rats chronic hypertension was induced by removing the right kidney without interfering with the left kidney. As controls, the left kidneys of 5 rats in which ligation of the right kidney failed to produce hypertension were used.

This study was aided by a grant from the John and Mary Markle Foundation.

From the Department of Pathology, University of Oklahoma School of Medicine, and the Department of Experimental Medicine, Southwestern Medical College.

1. Halpert, B., and Grollman, A.: Proc. Soc. Exper. Biol. & Med. **62**:273, 1946.

2. Grollman, A.: Proc. Soc. Exper. Biol. & Med. **57**:102, 1944.

Blood pressure determinations were made on the unanesthetized animals by the plethysmographic method of Williams, Harrison and Grollman.[3] Blood pressure recordings were made throughout the period of observation. All the animals used had chronic elevation of blood pressure for ten weeks or longer and appeared to be free of infection. The rats were killed after their blood pressure had been maintained at mean levels between 160 and 200 mm. of mercury for periods varying from ten to twenty weeks. The pertinent data concerning the mode of induction, the mean level of blood pressure and the duration of hypertension are recorded in the accompanying table.

Mode of Induction, Mean Level and Duration of Hypertension

Rat	Condition of Kidneys		Mean Blood Pressure, Mm. of Mercury	Duration of Hypertension, Weeks
	Right	Left		
43	Ligated	Intact	160	10
44	Ligated	Intact	180	12
45	Ligated	Intact	190	14
46	Ligated	Intact	200	16
47	Ligated	Intact	160	12
49	Ligated	Intact	180	13
50	Ligated	Intact	190	15
51	Ligated	Intact	170	14
52	Ligated	Intact	180	15
38	Ligated	Ligated	170	15
39	Ligated	Ligated	190	16
41	Ligated	Ligated	200	18
40	Ligated	Ablated	180	12
31	Ablated	Intact	200	20
48	Ablated	Intact	180	14

Microscopic studies were made of organs fixed in solution of formaldehyde U.S.P. diluted 1:25 embedded in paraffin and stained with hematoxylin and eosin. The kidneys and the hearts of the 15 rats with chronic hypertension were studied, and in 5 animals the lungs, the spleen and the liver and occasionally other organs (pancreas, intestine, mesentery, adrenal, brain) were also examined.

OBSERVATIONS

The hearts of all of the 15 rats with hypertension disclosed varying degrees of hypertrophy of the left ventricle. In fact, the hypertrophic changes as objective criteria paralleled the degree and the duration of the hypertension. The increase and the variation in size of the myocardial fibers are well illustrated in figure 1*A* from rat 40 in which the hypertension was induced by a figure of eight ligation of the right kidney and subsequent operative removal of the left kidney. The mean blood pressure at the end of twelve weeks was 180 mm. of mercury. In figure 1*B* are shown some of the changes occurring in the right kidney.

3. Williams, J. R., Jr.; Harrison, T. R., and Grollman, A.: J. Clin. Investigation **18**:373, 1939.

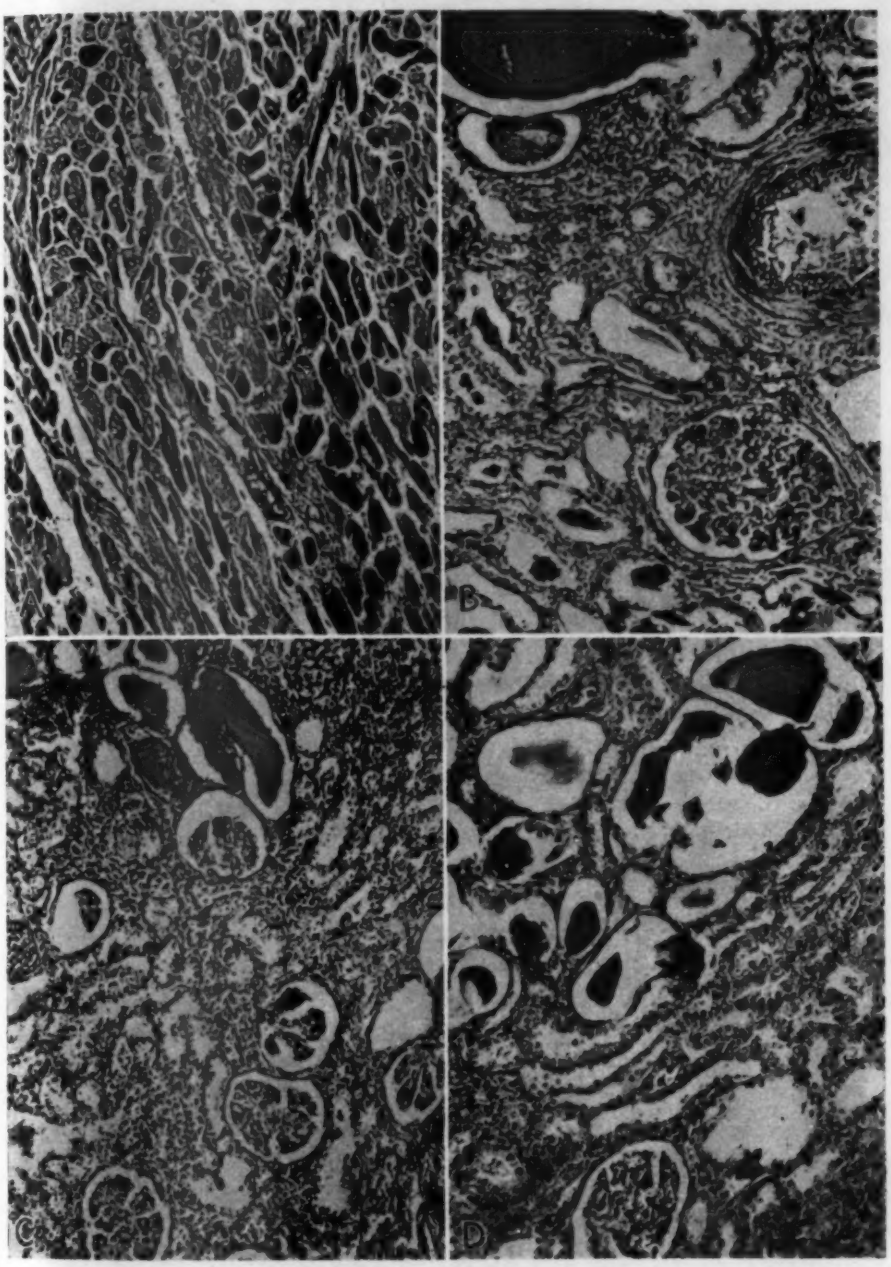


Fig. 1.—*A*, changes in the myocardium of the left ventricle and *B*, changes in the right kidney of rat 40, in which hypertension was induced by a figure of eight ligation of the right kidney and subsequent operative removal of the left kidney. × 100. *C* and *D*, comparable changes in the right and left kidneys of rat 44, in which hypertension was induced by a figure of eight ligation of the right kidney, the left being left unmolested. The cellular reaction in the right kidney is toward the ligature. × 100.

In this series as in the series of rats previously reported, the blood vessels of all of the organs examined revealed minimal or no changes.

In the 9 rats (table) in which a figure of eight ligature was applied to the right kidney, the left being left intact, the changes observed in both kidneys were essentially the same as those previously reported. The right kidneys, to which the ligatures were applied, were decreased in size, with deep fissures subdividing the surface. On the cut surfaces the widths of the cortical and medullary zones were decreased in proportion to the damage of the renal parenchyma. The cotton thread with which the kidneys had been constricted was demarcated microscopically by a zone of connective tissue infiltrated with lymphocytes, plasma cells and large mononuclear cells. The last often contained brown granules. Occasional giant cells of the foreign body type were also seen. The nearby renal parenchyma was transformed into scar tissue. The changes in the glomeruli ranged from a slight focal fusing of the capillaries with Bowman's capsule to complete disappearance of the capsular space and fibrous connective tissue replacement of the glomerulus with varying degrees of hyaline change. The convoluted and collecting tubules of such glomeruli were completely obliterated or were distended with a bright pink homogeneous material (figs. 1*C* and 2*A*).

The damage of the renal parenchyma of all of these kidneys was marked and was estimated to involve over half of the nephrons. The degree of involvement varied but was usually most marked about or near the course of the constricting thread. Occasional islands of fairly intact renal parenchyma were seen between the injured nephrons or their remains. In none were there any obvious changes in the large or smaller intrarenal blood vessels.

The left unmolested kidneys of these 9 rats were not distorted grossly. The microscopic changes were focal and involved an occasional single glomerulus or groups of glomeruli. They consisted of varying degrees of obliteration of the glomerular pattern. The convoluted and collecting tubules had coarse convolutions, contained a homogeneous bright pink material and were lined by flat cells. They occupied spaces at times two or more times the diameter of an intact glomerulus (figs. 1*D* and 2*B*). Here, too, no obvious changes were seen in the blood vessels.

A comparison of the changes in the right ligated and the left unmolested kidneys of this group of rats (figs. 1*C* and *D* and 2*A* and *B*) suggested that the two kinds of changes, one inducing the hypertension and the other produced by the hypertension, could be identified with reasonable certainty. The distorting coarse atrophy and scarring reducing the renal parenchyma along the constricting thread applied to the right kidney obviously were the lesions inducing the hypertension. The changes present in all of the left kidneys must be assumed to be

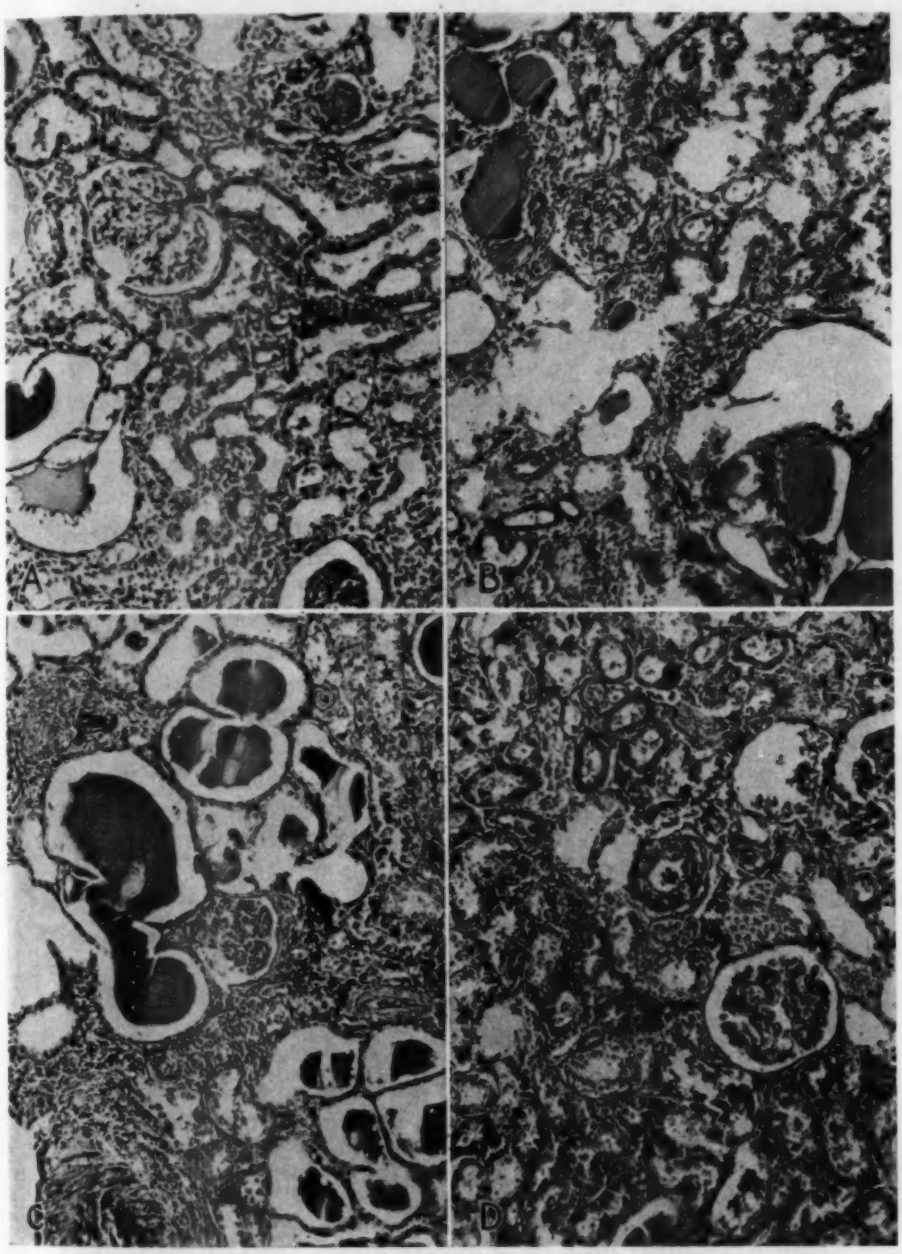


Fig. 2.—*A*, changes in the right and, *B*, changes in the left kidney of rat 46, in which the right kidney had been ligated, the left being unmolested. The changes in the left kidney, *B*, are assumed to be due to the hypertension. × 100. *C*, changes in the right and, *D*, changes in the left kidney of rat 41 in which a ligature was applied to the right kidney and subsequently one also to the left kidney. The changes in the right kidney are marked. The field chosen in the left kidney is relatively free of involvement. × 100.

due to the hypertension. The comparable changes observed in the right kidneys are also attributable to the hypertension.

The mechanism by which the hypertension injures the glomeruli is not clear. In both kidneys, however, the lesions attributed to the hypertension involved primarily the glomeruli themselves and then the tubules belonging to them. With the exception of the glomerular capillaries no changes were seen in any of the intrarenal blood vessels. Consequently it must be assumed that the changes in the nephrons were accomplished by injury of the intraglomerular capillaries.

In the 3 rats (table) in which a figure of eight ligature was applied to the right kidney without producing hypertension and three months later one was applied to the left kidney, changes were observed in both kidneys. The scarring and atrophy in both kidneys were similar to those seen in the right kidneys of the previous group. The changes in the right kidneys, however, seemed older than those in the left and were less extensive, leaving more uninvolved renal substance (fig. 2*C* and *D*). Changes produced by the hypertension as seen in the left unmolested kidneys of the first group were also seen in the right kidneys of these 3 rats.

In the rat in which the left kidney was removed subsequent to ligation of the right kidney the involvement of the right kidney was particularly extensive (fig. 1*B*). In this kidney there were few intact nephrons. Other nephrons were in all stages of injury: from recent obliteration of the glomerular pattern, where a pink-stained coagulum occupied the space inside Bowman's capsule with only ghosts of the capillaries remaining, to complete hyaline change of the glomeruli.

In the 2 rats in which hypertension was produced by removal of the right kidneys, the left kidneys were grossly not distorted. The microscopic changes were focal rather than diffuse and were quite similar to those seen in the left kidneys of the rats in which hypertension was induced by ligature of the right kidneys. Here, too, there were no obvious changes in the blood vessels other than those in the glomerular capillaries.

In the 5 control kidneys no gross changes were seen externally or on their cut surfaces. Microscopic examination disclosed good-sized glomeruli with intact convoluted and collecting tubules. No changes were seen in the stroma and blood vessels.

COMMENT

All the animals used in these experiments appeared to be free of infection. Animals manifesting acute illness or harboring suppurative lesions were considered unsuitable for the study of the effects of uncomplicated chronic hypertension and therefore were eliminated, since their use has led to unwarranted conclusions.

The hypertension in our experimental animals was apparently caused by damage or elimination of the renal parenchyma which reduced the number of available functioning nephrons. Such reduction was initiated by a figure of eight ligation of one or both kidneys or by ablation of one kidney without interference with the other. When chronic hypertension was once established, it caused further injury of the remaining nephrons whether one or both kidneys were present.

The induction of hypertension by unilateral nephrectomy, the opposite kidney not being interfered with, speaks against the assumption that a pressor agent liberated into the circulation from injured renal tissue is responsible for the development of the observed hypertension.[4]

The results of the present study support the view that neither damage of renal tissue nor ischemia nor intrarenal vascular changes are primary prerequisites for the induction of hypertension. It would rather appear that reduction of the total number of functioning nephrons is responsible for the development of the disorder. Hypertension once established damages additional nephrons, which further increases the hypertension. Therefore in the rat as in man it is a progressive disease the advance of which may be retarded but not abolished.[5]

SUMMARY

Experimental chronic hypertension was produced in 15 rats by damage or elimination of renal parenchyma which reduced the number of available functioning nephrons. Such reduction was initiated when a figure of eight ligature was applied to one or both kidneys or when one kidney was removed without interference with the other. The chronic hypertension thus produced resulted in hypertrophy of the left cardiac ventricle which paralleled the degree and the duration of the hypertension.

The data presented support the view that neither damage of renal tissue nor ischemia nor intrarenal vascular changes are primary prerequisites for the induction of hypertension but that it is the reduction of the total number of functioning nephrons which initiates the disorder.

Hypertension once established caused further injury to the remaining nephrons whether one or both kidneys were present. Therefore, chronic hypertension of the rat is a progressive disease, the advance of which may be retarded but not abolished.

---

4. Grollman, A.; Harrison, T. R., and Williams, J. R., Jr.: Am. J. Physiol. **139**:293, 1943. Loomis, D.: Arch. Path. **41**:231, 1946. Grollman, A., in Pincus, G.: Recent Progress in Hormone Research: Proceedings of the Laurentian Hormone Conference, New York, Academic Press, Inc., 1947, vol. 1, p. 371.

5. Grollman, A., in Goldring, W., and others: Experimental Hypertension, Special Publications, New York Academy of Science, 1946, vol. 3, p. 99.

THE FURTHER EFFECT OF THE LEUKOCYTOSIS-PROMOTING FACTOR OF EXUDATES WHEN INJECTED IN CONNECTION WITH INFLAMMATION

VALY MENKIN, M.D.
PHILADELPHIA

IN STUDIES previously reported there was demonstrated in exudative material the presence of a factor capable of reasonably explaining the leukocytosis frequently accompanying inflammatory processes.[1] This factor when injected into animals not only induces a discharge of immature granulocytes of the bone marrow but produces a hyperplasia or growth of granulocytes and of megakaryocytes in the marrow.[2] Its activity in human beings suggests that the material may have a clinical application.[3] It is well known that the prognosis of many infectious processes is to a large extent referable to the number of circulating leukocytes. If, therefore, with a given local inflammation the leukocyte level could at will be raised for a protracted period, a definite tool would be available to one in reenforcing antibiotics when dealing with a number of infectious processes.

In this communication data are collected to indicate that, with an intravascular injection of the leukocytosis-promoting factor (abbreviated as the LPF) and a concomitant pleural inflammation, the high level of leukocytes is maintained for longer intervals. The superimposition of this substance tends to reenforce the very mechanism which aids in the ultimate disposal of an irritant, namely, the rise in the number of circulating leukocytes.

EXPERIMENTS

Dogs were used and 1.5 cc. of turpentine was injected intrapleurally as described previously.[4] By the next day acute pleurisy had developed, and the white blood cell level was elevated. At that time various amounts of canine leukocytosis-promoting factor ranging from 23 to 57 mg. dissolved in several cubic centimeters of isotonic solution of sodium chloride were introduced into the

This paper is no. 38 of a series entitled "Studies on Inflammation."

From the Agnes Barr Chase Foundation for Cancer Research, Temple University School of Medicine.

1. Menkin, V.: Am. J. Path. **16**:13, 1940; Arch. Path. **30**:363, 1940. Menkin, V., and Kadish, M. A.: Am. J. M. Sc. **205**:363, 1943.
2. Menkin, V.: Am. J. Path. **19**:1021, 1943.
3. Menkin, V.: Arch. Path. **41**:376, 1946.
4. Menkin, V.: Am. J. Path. **10**:193, 1934.

circulation. Hourly counts were taken for about six hours to determine the level of the circulating white cells. A white blood cell count was taken once daily for each subsequent day until the level of circulating white cells was again approximately similar to the basal level prior to the beginning of the experiment. The results of the observations are assembled in table 1. It is clear that the intravascular superimposition of a dose of leukocytosis-promoting factor when there is a concomitant local acute inflammation increases markedly the level of circulating leukocytes. In the series of dogs studied the average basal white blood cell level amounted to 11,683. With an acute pleural inflammation, leukocytosis ensued, so that the average count was 29,329. With the intravascular injection of the leukocytosis-promoting factors, the leukocytosis became more pronounced; the average white blood cell count was 43,008. This additional activation of the marrow by the injected leukocytosis-promoting factor caused the white blood cell level to remain elevated for periods ranging from three to twenty-three days, with an average of nine days. On the other hand, when controls were used which

TABLE 1.—*The Effect of the Leukocytosis-Promoting Factor on the Level of Circulating Leukocytes with a Concomitant Local Inflammation*

Dog	Amount of LPF Injected into Blood at Time of Pleural Inflammation, Mg.	Basal White Blood Cell Count	White Blood Cell Level One Day After Development of Pleural Inflammation Alone	Maximal White Blood Cell Count Within Five Hours After Intravascular Injection of LPF at Time of Pleural Inflammation	Days Following Additional Intravascular Injection of LPF With Local Inflammation Before White Blood Cell Level Became Again Normal
38-T.....	23.0	8,650	27,950	39,300	23
30-T*.....	37.7	4,750	11,275	16,000	12
39-T.....	33.0	18,025	52,400	62,850	6
33-T.....	34.0	9,450	19,700	34,500	5
16-T.....	57.0	13,075	36,100	54,750	4
37-T.....	51.0	16,150	28,550	50,650	3
Average.....	39.3	11,683	29,329	43,008	9

* This animal had been given an intrapleural injection of the irritant about two months previously. This is the second injection of the irritant.

TABLE 2.—*The Effect of an Acute Local Inflammation of the Pleura on the Leukocyte Level in the Circulation*

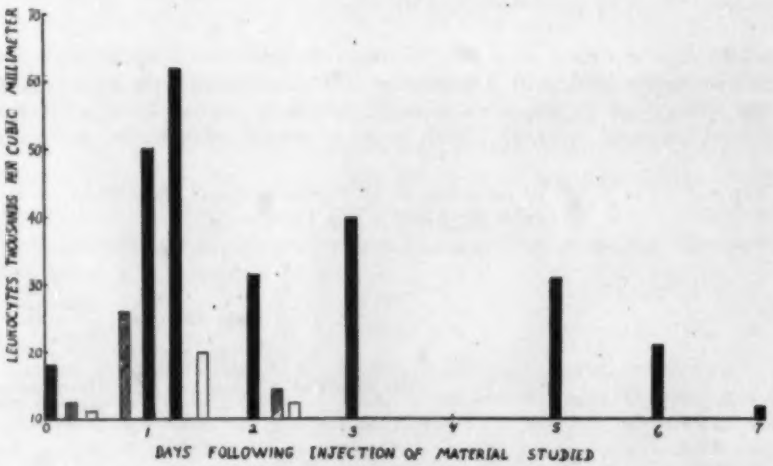
Dog	Basal White Blood Cell Count	White Blood Cell Level One Day After Intrapleural Injection of Irritant	Days Following Acute Inflammation of the Pleural Cavity Before White Blood Cell Level Became Again Normal
49-T.....	15,900	19,100	1
29-T.....	12,200	20,550	1
42-T.....	19,600	26,200	1
46-T.....	12,900	26,800	1
48-T.....	15,800	25,550	1
Average.....	15,280	23,640	1

received only the irritant (1.5 cc. of turpentine) in the pleural cavity, the leukocytosis failed to be sustained. About one day after the development of the acute pleural inflammation, the level of circulating leukocytes became again normal (table 2). This response is wholly different from the protracted effect seen when the administration of leukocytosis-promoting factor is added to an already existing inflammation (table 1). When the leukocytosis-promoting factor alone is given

TABLE 3.—*The Effect of a Single Intravascular Injection of Leukocytosis-Promoting Factor Alone on the Leukocyte Level in the Circulation*

Dog	Amount of LPF Injected into Blood, Mg.	Basal White Blood Cell Count	Maximal White Blood Cell Count Within 3 to 6 Hours After LPF Was Injected into Circulation	Days Following Intravascular Injection of LPF Before White Blood Cell Level Became Again Normal
9-T*	36	9,350	17,100	1
11-T*	51	11,000	21,100	1
8-D*	57	9,700	14,500	1
44-T	53	7,700	12,350	1
38-T	25	10,050	27,450	1
Average	44.4	9,500	18,500	1

* This animal had been given numerous injections of LPF in the past.



Graphic representation of the effects on the blood leukocyte count of: intravascular injection of leukocyte-promoting factor plus inflammation of pleura caused by intrapleural injection of turpentine (black columns); pleural inflammation alone (shaded columns); injection of leukocytosis-promoting factor alone (white columns).

to dogs, the level of circulating leukocytes remains elevated also for about one day (table 3). Typical experiments are illustrated in the accompanying figure. It is clear that when there is an inflammation in an animal and when in addition the leukocytosis-promoting factor is injected into the circulation, the number of circulating leukocytes is augmented for a period of about a week (figure), in

contrast to the short periods of elevation when there is either an inflammation alone or when the leukocytosis-promoting factor is injected only by itself. In such cases the number of circulating leukocytes remains high for approximately one day after the development of marked acute inflammation (figure). Observations were also made on 2 dogs which had previously received the leukocytosis-promoting factor and which several days later were given an intrapleural injection of turpentine. In these animals it seemed as if the activation of the bone marrow by the preliminary injection of the leukocytosis-promoting factor induced also a sustained response in the leukocyte level with the subsequent administration of the irritant. The counts in these 2 animals became normal only after four days. Thus, it would seem as if the leukocytosis-promoting factor administered either prior to the inflammation or after it is already in progress causes a rise in the leukocyte level which is maintained for several days longer than when there is just inflammation.

COMMENT

The foregoing observations clearly indicate that the number of circulating leukocytes can be increased and sustained at a high level when to an already existing inflammation an intravascular injection of leukocytosis-promoting factor is added. The combination of inflammation and injection of leukocytosis-promoting factor reenforces the natural leukocytosis which tends to develop with some types of inflammatory reaction. Since the prognosis of a number of inflammatory processes depends to some extent on the level of leukocytes [5] and since the leukocytosis-promoting factor can be injected innocuously into human beings,[5] herewith lies a factor which can be utilized in numerous clinical conditions in order to reenforce the antibiotics commonly used against various infectious processes.

SUMMARY AND CONCLUSIONS

The leukocytosis-promoting factor when injected into the blood stream of an animal with an already existing inflammation raises and maintains for a prolonged interval the number of circulating leukocytes. Under such circumstances the leukocytosis is sustained for several days longer than when there is an inflammation alone or when the leukocytosis-promoting factor is introduced without a concomitant inflammation. Some observations suggest also that this factor when injected several days prior to an acute inflammation of the pleura likewise tends to maintain a high leukocyte level in the blood for longer intervals. The clinical implications of these findings are discussed.

5. Robertson, D. H., and Fox, J. P.: J. Exper. Med. **69**:229, 1939.

EPIDERMOIDS (CHOLESTEATOMAS) OF THE BRAIN

Y. M. BHENDE, M.D.
AND
R. G. DHAYAGUDE, M.D.
BOMBAY, INDIA

NEITHER the name "pearly tumor" (*tumeur perlée*), given to these neoplasms by Cruveilhier [1] because of their highly refractile and nodular external surface, nor "cholesteatoma," as Müller [2] called them for their cholesterol content, is satisfactory. Histogenesis offers a firmer basis for correct nosology. The cholesteatoma occurring in the middle ear (air cells of the mastoid process) is, according to most, a legacy of chronic inflammation and, histologically, is a mass of laminated eosinophilic material devoid of cellular elements. But Cushing [3] believed even this to be a true epidermoid tumor which predisposed to secondary otitis media. Rand and Reeves [4] recently suggested the possibility that tumors of this type may be examples of the larger class of diploic or cranial epidermoids. Cholesteatomas occurring in situations other than the middle ear are, by a general consensus, considered genuine neoplasms. Histogenetically, they are either epidermoids (the commoner type) or dermoids arising from ectopic fetal epidermal cell rests (Bostroem [5]; Critchley and Ferguson [6]; Munro and Wegner [7]; Rand and Reeves [4]), a view originally put forth by von Remak.[8] "Cholesteatoma" is therefore an unfortunate designation, and these neoplasms should be termed epidermoids or dermoids depending on whether histologically they show pure ectodermal structures or whether, in addition to the epidermal lining, derivatives of the mesodermal corium can also be identified. The unequivocal demonstration of persistent squamous epithelium is, according to Love and Kernohan,[9] a

From the Department of Pathology and Bacteriology, Seth Gordhandas Sunderdas Medical College.

1. Cruveilhier, J.: Anatomie pathologique du corps humain, Paris, J.-B. Bailliere, 1829, vol. 1, book 2, p. 341.
2. Müller, J.: Ueber den feineren Bau und die Formen der krankhaften Geschwülste, Berlin, G. Reimer, 1838, vol. 1, p. 50.
3. Cushing, H.: Surg., Gynec. & Obst. **34**:557, 1922.
4. Rand, C. W., and Reeves, D. L.: Arch. Surg. **46**:350, 1943.
5. Bostroem, E.: Centralbl. f. allg. Path. u. path. Anat. **8**:1, 1897.
6. Critchley, M., and Ferguson, F. R.: Brain **51**:334, 1928.
7. Munro, D., and Wegner, W.: New England J. Med. **216**:273, 1939.
8. von Remak, R.: Deutsches Arch. f. klin. Med. **6**:170, 1854.
9. Love, J. G., and Kernohan, J. W.: J. A. M. A. **107**:1876, 1936.

point of distinction between a true neoplasm and an inflammatory cholesteatomatous mass. Characteristically the epidermoids are composed of four concentric layers of varying thickness—the acellular stratum durum responsible for the pearly shine, the cellular stratum granulosum made up of cuboidal to stratified epithelium containing keratohyaline granules within the cytoplasm of its cells, the stratum fibrosum and the stratum cellulosum. The breaking down of keratin and keratohyalin produces the cholesterol crystals. The dermoids, on the other hand, show an outer fibrous layer—a connective tissue matrix which may contain sebaceous glands, hair follicles, fat cells, smooth muscle fibers, elastic tissue fibers and blood vessels—and an inner epithelial layer.

These neoplasms—the epidermoids and the dermoids—have been shown to originate beneath the scalp, within the diploe of the skull, in the meninges, in the substance of the brain and the spinal cord, and even from the choroid plexuses of the ventricles. The commonest site is the subarachnoidal cistern, near the midline, at the base of the brain, or in the region of the fourth ventricle. For the epidermoid a common site is the cerebellopontile angle (Rand and Reeves [4]). The epidermoids are usually of a solid consistency, but the dermoids are more apt to be cystic. Both of them usually occur singly, grow slowly (the average duration of symptoms being many years) and run a benign course. Recurrence is mostly due to incomplete resection of the wall, but at least 2 cases in which there was a secondary malignant change have been recorded; in Ernst's [10] case a metastasizing carcinoma was the end result, and in Stromeyer's [11] case a sarcoma arose from the mesodermal element of the capsule. The symptoms and signs produced are varied and nonspecific; situated within the diploe the growth may produce a deformity and often a diagnostic roentgenogram, but if it occurs within the cranium, the symptom complex is that of increased intracranial tension, and the localizing signs are due to the effects of its pressure on the neighboring structures. There is no record of a single case of intradural epidermoid diagnosed prior to operation or autopsy (Love and Kernohan [9]). Not a few of these neoplasms have been accidental discoveries at the time of a carefully performed autopsy.

Verattus [12] described the first case of "cholesteatoma" (dermoid) of the brain, and up to 1936 some 56 cases of intracranial dermoid had been described (Courville and Kimball [13]). The epidermoid occurs more frequently. Rand and Reeves [4] computed that fewer than 200

10. Ernst, P.: Verhandl. d. deutsch. path. Gesellsch. **15**:226, 1912.

11. Stromeyer, F.: Beitr. z. path. Anat. u. z. allg. Path. **47**:392, 1909-1910.

12. Verattus, M. V.: De Bononiensi scientiarum et artium instituto atque academia commentarii, Bononiae, 1745, vol. 2, pt. 1, p. 184.

13. Courville, C. B., and Kimball, T. S.: Bull. Los Angeles Neurol. Soc. **1**:84. 1936.

cases of epidermoid of the central nervous system had been reported to date. The *Quarterly Cumulative Index Medicus* has not recorded any more cases of "cholesteatoma" of the brain to the end of 1945. It appears, therefore, that the so-called cholesteatoma of the brain is an uncommon neoplasm, accounting for less than 1 per cent of all kinds of intracranial growths.

REPORT OF CASES

CASE 1.—A Hindoo man aged 35 was admitted for (1) inability to use the left arm and the left leg—duration two years—and (2) headache—duration two months. The complaint started about two years prior to admission, with a feeling of weakness of the left foot. Gradually the weakness crept up to involve the whole of the limb. For the last several months he had used a stick to support himself, and for the last two months the limb had been practically useless. About six months after the onset of the first symptom he noticed weakness of the left hand, and this slowly spread up to involve the whole extremity. There was no subjective sensory disturbance of any kind in the affected limbs at any time. For the last two months he had suffered from headache in the frontal region. This was bilateral from its inception and in the beginning was irregular, with intervals of freedom, but for the last three weeks it had grown in intensity and had become constant. There was no history of vomiting, nor had he noticed any diminution of vision. As far as he remembered he suffered from no serious illness in his childhood. About six years ago he had a sore on the penis, which subsided without any specific treatment. He was a sweet-meat seller, subsisted on a vegetarian diet and smoked moderately; he had never tasted alcohol. There was nothing to note in the history of his family; he had five children, all healthy.

The general examination showed him to be a fairly well built and well nourished person. His temperature was 97 F.; pulse rate, 80; respiratory rate, 24. The conjunctivas and the nails were normal; the tongue appeared raw at the edges. The cervical lymph nodes of the right side were palpable, as also was the right epitrochlear gland; the inguinal nodes of both sides were palpable. Examination of the alimentary, respiratory and cardiovascular systems showed nothing abnormal. The blood pressure was 110 systolic and 70 diastolic. Psychologically he appeared to be a man of average intelligence and good memory. The neurologic observations were as follows: The pupils were central and equal but dilated; the reaction to light as well as to accommodation was sluggish. There was complete paralysis of the left side of the face; the other cranial nerves were normal. On the left side the motor power was diminished in both the upper and the lower extremities, more so in the latter. It was normal on the right side. The tone was increased on the left side, more so in the lower extremity. The right side appeared normal. The coordination, as far as could be judged, was unaffected. The left arm alone showed some wasting of the muscles; no test was made for the reaction of degeneration. The deep reflexes could be elicited on both the sides; all of those of the left side were markedly brisk. There was no patellar or ankle clonus. Among the superficial reflexes, the abdominal ones were present except in the left lower quadrant. The cremasteric reflex was absent on the left side. The plantar was flexor in type on the right side; on the left side it was extensor. The visceral reflexes were unaffected. The sensorium was normal. The gait was hemiplegic. Romberg's sign was observed.

Funduscopy showed bilateral papilledema. The field of vision of each eye was normal. Roentgenologic examination of the skull produced nothing distinctive.

Encephalography revealed defective visualization of the anterior horn of the right lateral ventricle. Ventriculography confirmed this finding: The right lateral ventricle was irregular, smaller and indistinct in outline; the left lateral ventricle was well seen but appeared displaced to the left.

Laboratory Investigations.—The blood revealed moderate normocytic, normochromic anemia. The leukocytic count was within the normal range. Both the Kahn and the Wassermann test were negative. The blood group was O.

The cerebrospinal fluid was under tension; manometry was not done. The fluid was clear. The total protein content was 200 mg. per hundred cubic centimeters; a test for globulins was positive; the sugar content was 60 mg. and the chloride content 680 mg. per hundred cubic centimeters; the cell count showed 3 cells per cubic millimeter, all lymphocytes; the Wassermann reaction was negative.

Gastric analysis showed a hypochlorhydric type of curve. The urine and the feces showed no abnormality.

Further Progress in the Wards.—The clinical diagnosis was "intracranial growth of the cerebrum, right side." An operation for decompression was undertaken. At operation the membranes were seen to be tense, and on incision the brain tissue protruded. The gyri were flattened, and the brain substance underneath felt firm. On incising the covering brain matter, one could see the neoplasm in the depths: it appeared as glistening white tissue, practically avascular and friable. The lesion appeared to be too extensive for any radical procedure, and only a small piece was removed for biopsy. The patient was in a state of severe shock after the operation. For this he was treated by injection of dextrose-saline solution and plasma and a blood transfusion. He was in a semiconscious state for two days and became deeply unconscious on the third day. His temperature shot up to 104 F., and he died on the fourth day after the operation. Permission to perform a full autopsy was refused, but permission to remove the brain was granted.

The Specimen (fig. 1).—The brain weighed 1,610 Gm. The membranes were normal except in the field of the surgical procedure. On separating them one saw that the gyri were flattened. The vessels on the surface of the brain were hyperemic. The organ was asymmetric and deformed; the right hemisphere of the brain was more bulky than the left one. The lesion appeared to be confined to the cerebral hemispheres; the cerebellum, the pons and the medulla were normal. The cut surface showed the following features: The neoplasm formed a large irregular mass measuring 8.5 by 8 by 6.5 cm. in the longest diameters. The greater part of it was situated centrally and extended into the cerebral hemispheres at the sides—to within 3 cm. of the left lateral surface of the cerebrum and to within 1.5 cm. of the right. Serial sections showed that superiorly on the right side it had nearly come to the surface, but that on the left side a width of 3 cm. of brain substance still lay intact. Inferiorly in the parieto-occipital region it had reached the deepest—to within 2 cm. of the base of the brain. Viewed as a whole, the bulk of it lay in the parieto-occipital region, but a small offshoot, measuring 2 by 1.5 cm., projected into the right frontal lobe. A membranous capsule could be traced all round, and with the naked eye one could see no definite infiltration of the brain substance at its margins. The impression produced was that the neoplasm had hollowed out for itself a bed in the substance of the brain tissue. In this process it had encroached on the major portion of the right lateral ventricle, leaving just a small part of the anterior horn still intact. On the left side, the body of the lateral ventricle alone was occupied, and the anterior and inferior

horns were free. All the structures in the wide compass of its extent were, naturally, destroyed. The neoplasm was apparently soft and friable, because many bits from it were easily dislodged and fell off during the sectioning. The cut surface was pale grayish white and grayish yellow; at many places were specks and streaks and even small irregular islets of tissue, which were glistening and pearly looking. The matter composing the neoplasm formed large irregular flakes, held loosely together. The brain substance all round the neoplasm was evidently compressed and both the cerebral hemispheres irregularly deformed. It may be stated, in retrospect, that the biopsy material sent for examination had sufficient distinctive features like the parent neoplasm to suggest its cholesteatomatous nature, and the scraping made from it and examined as a cover slip preparation showed cholesterol crystals.

Histologic Examination (fig. 2).—Most of the tissue consisted of cholesteatomatous matter in which coarse strands showed eosinophilic staining and were disposed in a wavy manner. No cellular elements could be detected in this. Clefts of cholesterol crystals could be seen all over. A large number of blocks had to be cut from different portions of the neoplasm before the epidermal lining could be demonstrated. The latter showed all the different layers—the stratum durum, the stratum granulosum, the stratum fibrosum and the stratum cellulosum. None of the sections showed any derivatives of the corium.

Case 2.—A Hindoo man aged 25 years complained of pain in the left half of the face—duration six months. The onset was insidious, with pain localized to the angle of the mouth on the same side in the beginning. The pain was, according to him, deep seated, growing, irregular in occurrence and exacerbated by any movement of the part. It had gradually increased in intensity and spread over a wider area to involve in turn the inside of the mouth, the lower jaw, the face and lastly the scalp. All along, it had been strictly confined to the left half of the face. For the last two weeks it had become excruciating and of a lightning-like character; it was now made worse by the slightest movement of the face and was persistent, with no intervals of freedom. There was no history of headache or vomiting. There was nothing significant in his past history or in the family's history. He was a man of moderate habits and earned his bread at a clerical job.

At this stage—after suffering for about six months—he consulted a surgeon outside the hospital. The relevant details extracted from the surgeon's notes are as follows: The patient was in extreme agony at the time of examination. The only physical sign to note on local examination was the tenderness all over the left half of the face. Examination of the nervous system gave negative results. The other body systems were normal. Funduscopy was not done. The surgeon's diagnosis was "trigeminal neuralgia." Six days before the patient sought admission to the hospital the surgeon attempted to inject alcohol into the trigeminal ganglion. Within a few minutes after the injection there developed marked swelling of the lids of both eyes, redness of the left half of the face and bilateral ptosis. Both pupils were dilated, and there was no reaction to light or accommodation. The pain continued unabated as before. He was immediately transferred to the King Edward VII Memorial Hospital, Bombay.

Examination on admission showed a well built and fairly well nourished man. His temperature was 99 F.; pulse rate, 110; respiratory rate, 26. The look was anxious, and the man appeared to be overwhelmed by the pain. The eyelids of both eyes were moderately swollen, and the skin over the left half of the face was reddened. The left eye alone showed ptosis, and its upper eyelid was redder than the rest of the face on that side. The left pupil was semidilated and reacted

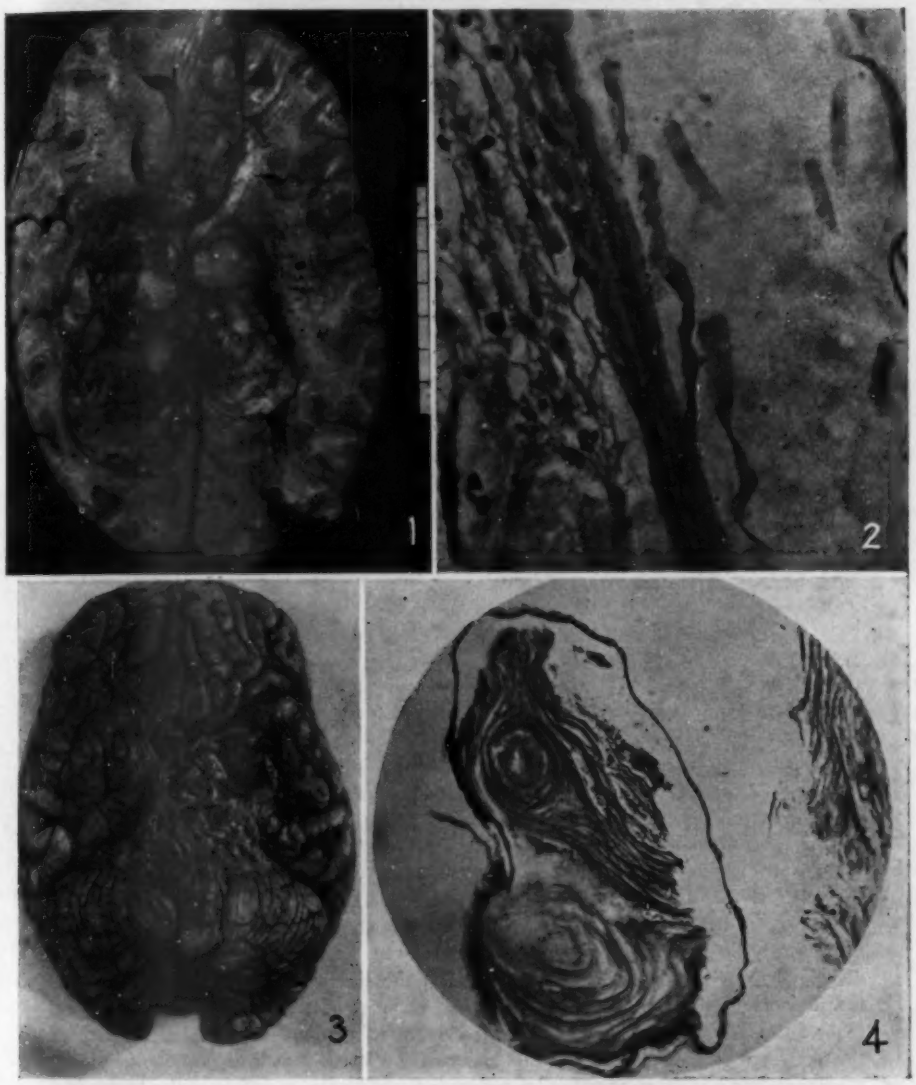


Fig. 1 (case 1).—Specimen of the brain—the cut surface of the lower half. It shows the extent and the general features of the neoplasm.

Fig. 2 (case 1).—Photomicrograph of a histologic section of the specimen. The stratum granulosum with the keratohyaline granules in its cells can be easily seen. × 200.

Fig. 3 (case 2).—Specimen of the brain showing the main mass of the neoplasm lying in the recess between the pons and the cerebellum, on one side, and the temporal lobe, on the other. The latter shows raw areas (operative injury).

Fig. 4 (case 2).—Photomicrograph of a histologic section of the specimen. Note the epidermal lining all round an islet of cholesteatomatous tissue. Hematoxylin and eosin; × 70.

sluggishly to light; the reaction to accommodation was normal. The right pupil showed no abnormality. The left half of the face was tender all over. The sensations of touch, pain and pressure were clearly elicited over the right half of the face, but over the left half they seemed somewhat dulled. The rest of the nervous system was normal and the other body systems showed no changes. The dental surgeon reported the gums to be pyorrheic but could find no carious or tender teeth. An overhaul by the ear, nose and throat department revealed no abnormality. Unfortunately, even at this stage, funduscopy was not done.

Laboratory Investigations.—Cytologic examination of the blood gave a normal count. The Kahn test was positive. The urine and the feces showed no abnormalities. The cerebrospinal fluid was under tension. The chemical and cytologic examinations showed no deviations from the normal.

Further Progress in the Wards.—The clinical diagnosis was "trigeminal neuralgia." The pain continued, and it was decided to perform Frazier's operation of trigeminal ganglionectomy. The ganglion was successfully resected accordingly; nothing eventful was noticed at the operation. However, after the operation the patient never regained consciousness. He gradually became comatose, a high temperature of 105 F. developed and death occurred within twelve hours after the operation. The permission for autopsy allowed only removal of the brain.

The Specimen (fig. 3).—The brain weighed 1,250 Gm. The membranes were hyperemic all over. There was no flattening of the gyri or deepening of the sulci. The brain substance proper appeared redder than normal. There was no deformity of the cerebrum as seen from its superior and lateral surfaces. Examination of the base of the brain showed that the left temporal lobe was damaged. Here the surface was uneven, at places raw, and large blood clots were clinging to the brain tissue. After these had been gently removed, the brain substance beneath was found depressed and flattened. In addition, there could be seen now a grayish white and grayish yellow refractile tissue distributed irregularly over the inferior surface of the left temporal lobe and the left half of the pons along its inferior and lateral surfaces. The major portion of this mass was, however, lodged in the recess between the pons and the cerebellum, on one hand, and the adjoining portion of the left temporal lobe, on the other. The exact extent of this mass was difficult to decide; its original relationship appeared to have been disturbed by the operative procedures. It formed, however, an irregular, lobulated mass measuring 4 by 3 by 2 cm. in its widest diameters. It seemed to be situated intradurally in the subarachnoid space. It was friable, small bits being easily dislodged even by gentle handling. Both in the fresh and in the fixed specimen, the impress of the tumor on the adjoining surfaces of the pons and the temporal lobe of the left cerebral hemisphere was evident. The disposition and the extent of the neoplasm as judged even from its remnants indicated that it could have produced the interesting symptom complex by pressure either on the ganglion of the trigeminal nerve or on the sensory root of the trigeminal nerve.

Histologic Examinations (fig. 4).—Most of the material consisted of cholesteatomatous matter. After a prolonged search of the sections from different portions, one bit was found to show the epidermal lining. This formed a thinned layer of three to five rows of cells; the stratum durum and the stratum granulosum could be identified.

Case 3.—A Hindoo man aged 21 years was admitted with the history of having met with a serious tramcar accident. He was in a state of advanced surgical shock. There was a compound fracture of the bones of the left leg and a crushing injury of the right foot. He died within three hours after admission.

Autopsy.—All the injuries mentioned in the clinical notes were confirmed. Beyond the changes in viscera, produced by shock, there was nothing to note. The brain showed at its base a small neoplasm. This was situated intradurally and was adherent by a tiny tag to the right cerebellar peduncle and in the same way to the petrous portion of the temporal bone. The whole organ was carefully dissected out and fixed.

The Specimen (fig. 5).—The brain weighed 1,200 Gm. The meninges were normal. The gyri and the sulci did not show changes. At the base, in the right cerebellopontile angle, was seen an irregular, freely mobile mass measuring 3 by 2.5 by 2 cm. It seemed to lie in the subarachnoid space. It was well circumscribed and covered by a thin capsule. The brain substance was quite free. The under surface of the cerebellum was slightly indented by the mass, and its impress on the lateral surface of the pons was even more shallow.

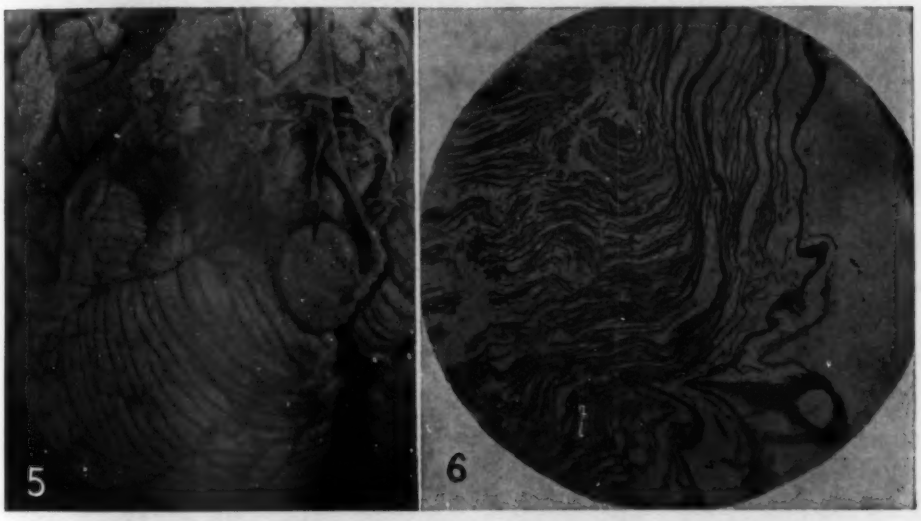


Fig. 5 (case 3).—Specimen of the brain showing the neoplasm lying in the right cerebellopontile angle.

Fig. 6 (case 3).—Photomicrograph showing the cholesteatomatous tissue with the epidermal lining on the surface. Hematoxylin and eosin; × 70.

Histologic Examination (fig. 6).—Most of the tissue was fibrillar and cholesteatomatous in nature. Many of the bits, however, showed a thinned out epidermal lining. This consisted of two to four layers of polyhedral cells with dark oval nuclei and eosinophilic nongranular cytoplasm. The outer surface of this lining showed keratinization at places, but nowhere could elements of the corium be demonstrated.

COMMENT

The unequivocal demonstration of an epidermal lining in all the 3 tumors would differentiate them as true neoplasms (Love and Kernohan [9]). No derivatives of the corium were detected, and histologically, therefore, the neoplasms were epidermoids. That in the first case had

grown to such a large size that its exact origin could not be told; its situation, however, suggested its seat to be some midline structure. The production of the characteristic symptoms of an "intracranial growth" could be easily appreciated. In the second case the neoplasm had produced pronounced pressure symptoms to delude the clinician into a diagnosis of trigeminal neuralgia. There was neither headache nor vomiting. The omission of a funduscopic examination was most unfortunate. This investigation might have been helpful in reaching the correct diagnosis, and in the absence of any data from this source it would be incorrect to state that the general symptoms of an "intracranial growth" were not present. The neoplasm in this case was situated in the subarachnoid space and was meningeal in origin. In the third case the neoplasm arose from the meninges and was discovered at autopsy. The history subsequently elicited, if relied on, would suggest that the growth had produced no symptoms during life.

SUMMARY

Three cases of epidermoid of the brain are reported.

In the first case the symptoms and signs of an "intracranial growth" were evident enough to enable one to reach the correct diagnosis. In the second case the neoplasm produced peculiar pressure symptoms, and the case was misdiagnosed as one of trigeminal neuralgia. In the third case the neoplasm was an incidental finding at autopsy.

NODULAR INFLAMMATORY AND DEGENERATIVE LESIONS OF MUSCLES FROM FOUR HUNDRED AND FIFTY AUTOPSIES

B. J. CLAWSON, M.D.

J. F. NOBLE, M.D.
AND
N. H. LUFKIN, M.D.
MINNEAPOLIS

IT HAS been an interesting observation that rheumatic and rheumatoid inflammations tend to be located in nodules in various tissues of the body. The terms "Aschoff nodules," "subcutaneous nodules," "Masson bodies" and "nodular polymyositis" are commonly noted in the medical literature.

Rheumatic nodular inflammation has been observed and described in the subcutaneous tissues,[1] the joints, the tendons, the diaphragm, the tongue and other muscles,[2] the galea aponeurotica,[3] the tonsils,[4] the arteries,[5] the valves,[6] atriums [7] and ventricles [8] of the heart and the lungs.[9] Subcutaneous nodules and similar inflammatory processes have repeatedly been seen and described in association with the state of rheumatoid arthritis.[10]

In 1928 Maclachan and Wayne [4] studied the tonsils and the muscles of the tongue and tonsillar region in cases of acute rheumatic

From the Department of Pathology, University of Minnesota.

This investigation was aided by a grant from the National Foundation for Infantile Paralysis.

1. Swift, H. F.: J. Exper. Med. **39**:497, 1924.
2. Graff, S.: Deutsche med. Wchnschr. **53**:708, 1927.
3. Jacki, E.: Frankfurt. Ztschr. f. Path. **22**:82, 1919-1920.
4. Maclachlan, W. W. G., and Wayne, G. R.: Ann. Int. Med. **1**:506, 1928.
5. Chiari, H.: Beitr. z. path. Anat. u. z. allg. Path. **80**:337, 1928. Klotz, O.: J. Path. & Bact. **18**:259, 1913.
6. Clawson, B. J.: Arch. Path. **8**:664, 1929.
7. MacCallum, W. G.: Bull. Johns Hopkins Hosp. **35**:329, 1924.
8. Aschoff, L.: Verhandl. d. deutsch. path. Gesellsch. **8**:46, 1904.
9. Neubuerger, K. T.; Geever, E. F., and Rutledge, E. K.: Arch. Path. **37**:1, 1944.
10. Dawson, M. H., and Boots, R. H.: J. A. M. A. **95**:1894, 1930. Clawson, B. J., and Wetherby, M.: Am. J. Path. **8**:283, 1932. Bennett, G. A.: Arch. Path. **30**:70, 1940.

fever. Between the muscles he found an infiltrate of lymphocytes, plasma cells and endothelial cells. When muscle fibers had become atrophic, they were invaded by endothelial leukocytes, and multinucleated giant cells were noted.

Curtis and Pollard [11] studied biopsy specimens of calf muscles in cases in which chronic arthritis was included in Felty's syndrome. They observed areas of interstitial and perivascular infiltration of lymphocytes.

Our interest in the character and the frequency of muscular inflammatory nodular areas and the degrees of muscular degeneration in cases of rheumatoid arthritis and acute rheumatic fever was stimulated by the recent thorough study of these muscular lesions by Steiner and his co-workers.[12] They studied the muscles in 9 cases of rheumatoid arthritis. The muscles had been obtained for biopsies. In the nodular inflammatory areas Steiner found lymphocytes and plasma cells to be abundant. Polymorphonuclear leukocytes and eosinophils were rare or absent. These nodules were located in the endomysium and the perimysium. In the larger nodules an epithelioid type of cell was seen. The nodules were found in each of the 9 cases.

In a study of 44 cases of rheumatoid arthritis with Wells and Wetherby,[13] biopsy specimens were obtained from deltoid muscles, and lesions were found similar to those described by Steiner, in 17 (38.6 per cent) of the cases.

Steiner also emphasized the degenerative processes which occurred in association with the nodular inflammatory areas. The degenerative changes noted in the muscles consisted of atrophy and various degrees of necrosis. Fatty metamorphosis and hydropic degeneration were noted. The muscular nuclei were increased in number, size and shape and stained more deeply than normal. It was Steiner's opinion that the degenerative changes were secondary to the inflammation. In 11 of our 44 cases one or more of degenerative processes were noted in the deltoid muscle.

In a control study of 196 specimens taken from routine autopsies the nodular myositis, the perineuritis and the degenerative nuclear changes were not noted by Steiner.

The purpose of the present paper is to furnish controls for our previous observations on biopsy specimens of muscles in cases of rheumatoid arthritis and to obtain further information concerning inflammation and degeneration in a greater number of cases and in a larger number of muscles than could be obtained by biopsies.

---

11. Curtis, A. C., and Pollard, H. M.: Ann. Int. Med. **13**:2265, 1940.

12. Steiner, G.; Freund, H. A.; Leichtentritt, B., and Maun, M. E.: Am. J. Path. **22**:103, 1946.

13. Wells, S. M., and Wetherby, M.: To be published.

Seven muscles were collected from each of 450 autopsies and studied for evidences of inflammation and various stages of degeneration. The muscles studied were the pectoral, the sternocleidomastoid, the deltoid, the diaphragm, the intercostal, the psoas and the sacrospinalis.

OBSERVATIONS

Inflammatory Lesions.—The nodular inflammatory areas were graded in respect to size and frequency from 1 to 4 plus. One plus

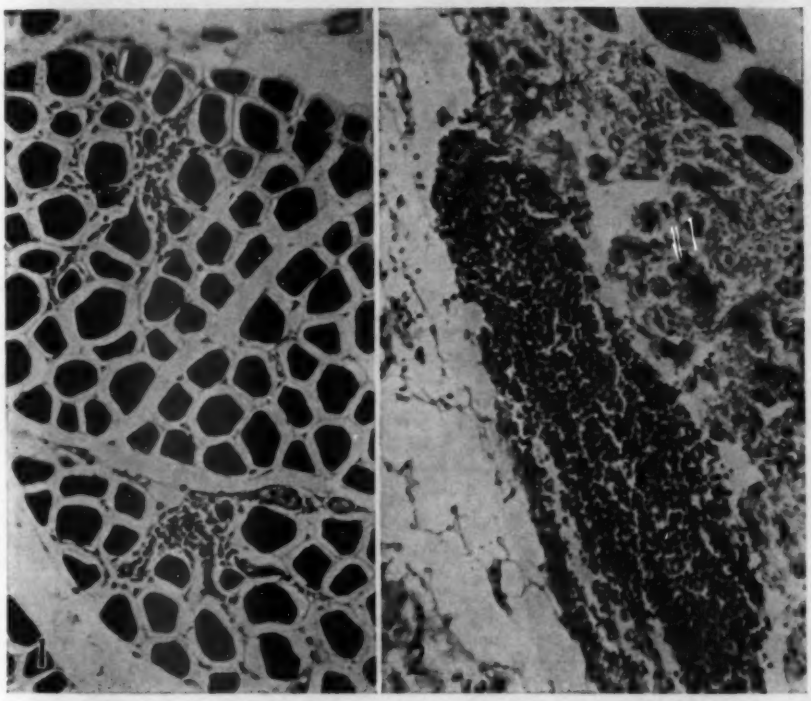


Fig. 1.—Inflammatory nodules (1 plus grade) between muscle fibers.
Fig. 2.—Inflammatory nodule (4 plus grade) in interstitial tissue.

represented a small nodule (fig. 1). The presence of but a few lymphocytes was not recorded. The presence of one or more nodules in a section was also considered in evaluating the grade. A 4 plus grade represented one or more large nodules or several smaller ones in the same section (fig. 2.). A 2 plus and a 3 plus grade were intermediate.

The inflammatory nodules consisted primarily of infiltrating lymphocytes and plasma cells. A few polymorphonuclear leukocytes were occasionally seen, and in a few cases they were the principal cells. In many of the nodules the cells were more or less oval in shape, and

definite macrophages were sometimes seen. Some of these resembled epithelioid cells. In a few cases carcinoma had metastasized to the muscles. In a case of lymphatic leukemia there was a diffuse infiltration of lymphocytes.

The frequency and the degree of the inflammatory processes in the various muscles in the 450 cases are recorded in table 1. In 118 (26.2

TABLE 1.—*Frequency and Degrees of Nodular Myositis (Four Hundred and Fifty Cases)*

Muscle	Cases Examined	Cases in Which Myositis Was Graded as Shown: +		++		+++		++++		+ or More	
		No.	%	No.	%	No.	%	No.	%	No.	%
1. Pectoral	450	10	2.2	7	1.5	0	...	0	...	17	3.7
2. Sternocleidomastoid	450	10	2.2	4	0.8	3	0.6	3	0.6	20	4.4
3. Deltoid	450	6	1.3	12	2.6	1	0.2	0	...	19	4.2
4. Diaphragm	450	16	3.5	25	5.5	14	3.1	6	1.3	61	13.5
5. Intercostal	450	10	2.2	16	3.5	5	1.1	5	1.1	36	8.0
6. Psoas	432	8	1.8	3	0.6	2	0.4	3	0.6	16	3.7
7. Sacrospinalis	150	4	2.6	5	3.3	0	...	0	...	9	6.0

Cases in which myositis of 1 plus or more was observed in one or more muscles—118 (26.2%)

TABLE 2.—*Frequency and Degrees of Atrophy of Muscles (Four Hundred and Fifty Cases)*

Muscle	Cases Examined	Cases in Which Atrophy Was Graded as Shown: +		++		+++		++++		+ or More	
		No.	%	No.	%	No.	%	No.	%	No.	%
1. Pectoral	450	14	3.1	3	0.6	0	...	1	0.2	18	4.0
2. Sternocleidomastoid	450	28	6.2	4	0.8	1	0.2	0	...	33	7.3
3. Deltoid	450	23	5.1	12	2.6	0	...	1	0.2	36	8.0
4. Diaphragm	450	90	20.0	34	7.5	1	0.2	0	...	125	27.7
5. Intercostal	450	33	7.3	4	0.8	1	0.2	0	...	38	8.4
6. Psoas	432	24	5.5	5	1.1	0	...	1	0.2	30	6.9
7. Sacrospinalis	150	16	10.6	3	2.0	2	1.3	0	...	21	14.0

Cases in which atrophy of 1 plus or more was observed in one or more muscles—191 (42.4%)

per cent) of the 450 cases inflammatory lesions were observed of one or more grades and in one or more muscles. The diaphragm and the sacrospinalis muscle were most commonly affected. As seen in table 5, there were 83 cases in which one muscle was involved; two muscles were involved in 24 cases, three muscles in 6 cases, four muscles in 2, five muscles in 1 and six muscles in 2. In no instance was there involvement of all seven muscles.

Degenerative Processes.—The degenerative processes consisted of atrophy, Zenker's degeneration, necrosis and an increase in the number and a change in the shape and the size of the muscular nuclei. The atrophic muscular fibers were markedly shrunken as compared with

normal fibers in the same section (fig. 3). The fibers showed various degrees of loss of striations. Some of the fibers were swollen with complete absence of both cross and longitudinal striations. Calcification of the fibers sometimes was noted. The sarcoplasm often was lysed and had completely disappeared. The nuclei were frequently greatly increased in number. They varied decidedly in size and shape and were hyperchromatic (fig. 4). These three degenerative changes were each graded 1, 2, 3 and 4 plus. The various degenerative processes

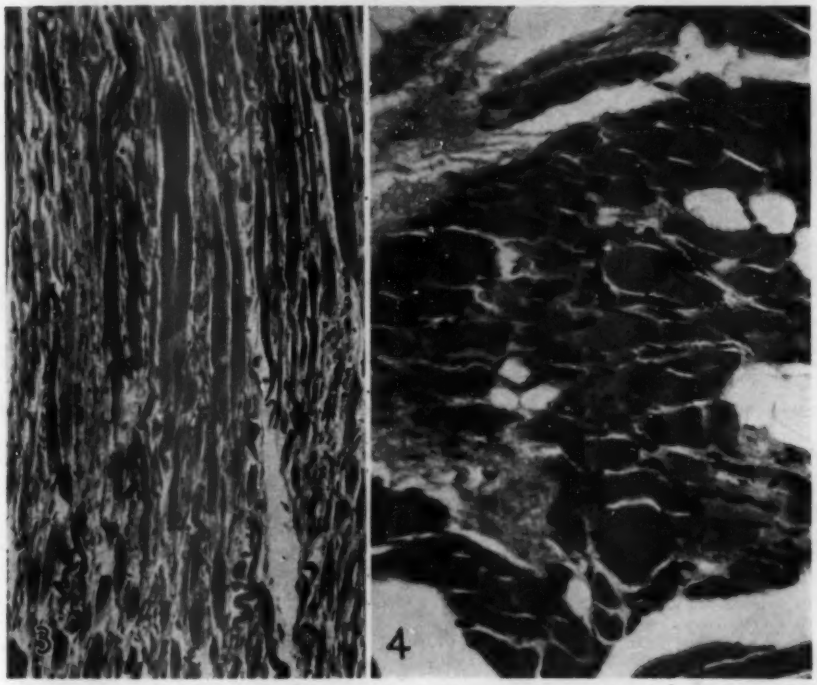


Fig. 3.—Atrophy of muscle with connective tissue replacement.
Fig. 4.—Nuclear enlargement and deformity.

observed in the muscles of the 450 cases reported in this paper are recorded in tables 2, 3 and 4.

Atrophy varying from slight narrowing to almost complete disappearance of the fibers was a common finding. The different degrees of atrophy and the frequency of involvement of the seven different muscles are recorded in table 2. Atrophy was present to some degree in all the muscles but was most pronounced in the diaphragm and the sacrospinalis muscle. In 191 of the 450 cases (42.4 per cent) there was atrophy of 1 plus or more in one or more muscles. The combination of muscular involvement per autopsy examined is recorded in table 5.

The frequency and the degrees of degenerative changes exhibited by cytoplasm varying from loss of striation to waxy degeneration and finally to complete necrosis of muscle fibers are tabulated in table 3. As with the inflammatory lesions, the greatest amount of change was noted in the diaphragm (17.5 per cent). The sternocleidomastoid muscle ranked next but was not much more commonly involved than the

TABLE 3.—*Frequency and Degrees of Sarcoplastic Changes in Muscles (Four Hundred and Fifty Cases)*

Muscle	Cases Examined	Cases in Which Sarcoplastic Changes Were Graded as Shown: +		++		+++		++++		+ or More	
		No.	%	No.	%	No.	%	No.	%	No.	%
1. Pectoral	450	5	1.1	2	0.4	1	0.2	0	...	8	1.7
2. Sternocleidomastoid	450	25	5.5	8	1.7	1	0.2	0	...	34	7.5
3. Deltoid	450	21	4.6	6	1.3	1	0.2	1	0.2	29	6.4
4. Diaphragm	450	62	13.7	13	2.8	4	0.8	0	...	79	17.7
5. Intercostal	450	21	4.6	2	0.4	1	0.2	0	...	24	5.3
6. Psoas	432	14	3.2	3	0.6	0	...	1	0.2	18	4.1
7. Sacrospinalis	150	3	2.0	2	1.3	1	0.6	0	...	6	4.0

Cases in which sarcoplastic changes of 1 plus or more were observed in one or more muscles—152 (33.7%)

TABLE 4.—*Frequency and Degrees of Muscular Nuclear Changes (Four Hundred and Fifty Cases)*

Muscle	Cases Examined	Cases in Which Nuclear Changes Were Graded as Shown: +		++		+++		++++		+ or More	
		No.	%	No.	%	No.	%	No.	%	No.	%
1. Pectoral	450	39	8.6	8	1.7	1	0.2	1	0.2	49	10.8
2. Sternocleidomastoid	450	32	7.1	6	1.3	1	0.2	0	...	39	8.6
3. Deltoid	450	32	7.1	19	4.2	1	0.2	1	0.2	53	11.7
4. Diaphragm	450	48	10.6	9	2.0	2	0.4	0	...	59	13.1
5. Intercostal	450	42	9.3	7	1.5	4	0.8	0	...	53	11.7
6. Psoas	432	25	5.7	7	1.6	2	0.4	0	...	34	7.8
7. Sacrospinalis	150	11	7.3	4	2.6	2	1.3	0	...	17	11.3

Cases in which nuclear changes of 1 plus or more were observed in one or more muscles—158 (35.1%)

other muscles with the exception of the pectoral muscle, which showed much less evidence of degenerative processes. In 152 of the 450 cases (33.7 per cent) there was atrophy of 1 plus or more in one or more muscles examined (table 5).

The nuclear changes observed showed several variations. The nuclei were often greatly increased in number. They varied in size and shape. Some became much elongated; some became enlarged and took on irregular shapes. The nuclei stained darkly.

The frequency and the degree of the nuclear changes (also graded 1, 2, 3 and 4 plus) of the seven muscles per autopsy examined are seen in table 4. The greatest amount of change, as with inflammation, atrophy

and sarcoplastic degeneration, was noted in the diaphragm (13.1 per cent); but the pectoral, deltoid, intercostal and sacrospinalis muscles were almost as commonly involved. Nuclear changes of 1 plus or higher grade were observed in one or more muscles in 158 of the 450 cases (table 5).

Correlation of the Clinical Histories and the Inflammatory Lesions.—In 312 of the 450 cases studied histories were available at the time of

TABLE 5.—*Combinations of Muscular Involvement*

Muscles Involved in Each Case	Cases in Which Given Abnormality Was Observed							
	Myositis		Atrophy		Sarcoplastic Changes		Nuclear Changes	
	No.	%	No.	%	No.	%	No.	%
1	83	70.33	126	65.96	116	76.31	79	50.00
2	24	20.33	39	20.41	26	17.10	41	25.94
3	6	5.08	13	6.80	5	3.28	17	10.75
4	2	1.69	6	3.14	4	2.63	13	8.22
5	1	0.84	3	1.57	0	....	5	3.16
6	2	1.69	2	1.04	0	....	2	1.26
7	0	....	2	1.04	1	0.65	1	0.63
Total	118	99.96	191	99.96	152	99.97	158	99.96

Cases in which one or more lesions were observed in one or more muscles—293 (65.1%)

TABLE 6.—*Myositis—Age and Sex Incidence (Three Hundred and Twelve Cases)*

Decades	Males			Females			Total		
	No.	No. with Myositis	% with Myositis	No.	No. with Myositis	% with Myositis	No.	No. with Myositis	% with Myositis
1	13	4	30.7	9	2	22.2	22	6	27.2
2	5	0	....	10	3	30.0	15	3	20.0
3	8	0	....	12	3	25.0	20	3	15.0
4	11	5	45.4	9	2	22.2	20	7	35.0
5	7	2	28.5	10	3	30.0	17	5	29.4
6	34	12	35.2	20	5	25.0	54	17	31.4
7	43	12	27.9	13	5	38.4	56	25	44.6
8	42	13	30.9	22	8	36.3	64	21	32.8
9	19	0	....	21	5	23.8	40	5	12.5
10	3	0	....	1	0	....	4	0	....
Total	185	48	25.9	127	36	28.3	312	92	29.4

the analysis. The age and the sex distribution and the types of diseases were studied in an attempt to determine whether any relation existed between any of these factors and the frequency and the degree of the nodular inflammatory lesions of the seven muscles of each case studied.

The age and sex incidence is recorded in table 6. The greatest number of males fall into the sixth, seventh and eighth decades and the greatest number of females into the sixth, seventh, eighth and ninth decades. The highest percentage of positive findings of inflammatory lesions is in the seventh decade in the entire group, but the percentage of positive findings is almost as great in the sixth and eighth decades.

The outstanding facts to be noted from this analysis of age and sex incidence are that the incidence of nodular myositis is about equal in the two sexes and that it is greater in both sexes in the later decades.

The types of disease mainly responsible for the deaths in the 312 cases analyzed and the relative frequency of myositis in each of the groups of diseases are listed in table 7.

TABLE 7.—*Myositis in Different Types of Diseases (Three Hundred and Fourteen Cases)*

Disease	Cases	No. with Myositis	% with Myositis
Heart diseases			
Acute rheumatic fever	6	6	100.0
Valve deformities	21	4	19.0
Bacterial endocarditis	5	2	40.0
Syphilis	1	0	....
Hypertension	24	7	29.1
Coronary sclerosis	31	8	25.8
Cor pulmonale	5	0	....
Total	93	27	29.0
Noninfectious diseases			
Accidents and trauma	43	11	25.5
Tumors	46	18	39.1
Cerebral hemorrhage	15	3	20.0
Cirrhosis of liver	9	5	55.5
Poisoning	5	0	....
Gastrointestinal conditions	11	5	45.4
Stillbirth or neonatal conditions	4	1	25.0
Rupture of aneurysm	3	1	33.3
Thrombosis or embolism	1	1	100.0
Diabetes	6	1	16.6
Anemia	1	0	....
Total	144	46	31.9
Infectious diseases			
Poliomyelitis	22	2	9.0
Tuberculosis	16	4	25.0
Infections of bladder and kidneys	14	2	14.2
Pneumonia	7	1	14.2
Abscesses	6	0	....
Peritonitis	4	1	25.0
Meningitis and encephalitis	2	0	....
Influenza	1	0	....
Lupus erythematosus	1	1	100.0
Bacteremia	1	0	....
Cholecystitis	3	1	33.3
Total	77	12	15.5

In table 7 are seen, first, the different diseases of the heart, with the percentage of cases of each in which there were inflammatory lesions of the heart muscle. There were 6 cases of acute rheumatic endocarditis in all of which inflammatory lesions were observed in one or more of the seven muscles. In only 4 of the 21 cases of valvular deformities resulting from previous rheumatic infections was there any evidence of myositis. In 2 of the 5 cases of subacute bacterial endocarditis there was myositis. There was only 1 case in which cardiac failure was due to syphilitic aortitis. No muscular lesions were observed in this case. In the hypertensive cases and cases in which death was

due to coronary sclerosis or thrombosis the frequency of muscular lesions was high, 29.1 per cent and 25.8 per cent, respectively. No muscular inflammatory lesions were present in the 5 cases of cor pulmonale. In 27 (29.0 per cent) of the 93 cardiac cases the nodular muscular lesions were present in some degree in one or more of the seven muscles examined. Two observations worthy of note in these cardiac cases are that muscular lesions are high in patients with acute rheumatic endocarditis, the young patients, and in patients with hypertension and coronary sclerosis, the older patients.

The second group of diseases shown in table 7 comprises noninfectious conditions. There are 144 cases. In 46 (31.9 per cent) the muscular lesions were observed. The incidence was high in patients dying of accidents and trauma, tumors, cirrhosis of the liver and gastrointestinal diseases. Nothing of relative importance is suggested in this group except that many of the positive findings are in the older people. The same was observed in the cardiac group.

A group of 77 cases in which infection of one kind or another, not including infectious heart diseases, was the chief cause of death is recorded last in table 7. In only 12 (15.5 per cent) of these was myositis present. The incidence was fairly high in cases of tuberculosis and in cases of renal infection (pyelonephritis, glomerulonephritis, etc.). An interesting observation is that muscular lesions were infrequent in the cases in which death was due to acute poliomyelitis; the patients were mostly young people. In the single case of lupus erythematosus six of the seven muscles were extensively involved. This group in general included younger people than the cardiac and noninfectious groups.

Association of Inflammatory and Degenerative Lesions.—Steiner, in his studies, found what he believed to be a definite relationship between nodular polymyositis and degeneration and atrophy of muscle fibers.

Of our 450 autopsies 118 disclosed inflammatory myositis. In 83 of these 118 cases (70.3 per cent) there was also one or more of the degenerative changes noted in the muscles. Degenerative processes were more common than inflammatory lesions. Degenerative muscular lesions (atrophy, cytoplastic changes or nuclear changes) were noted in 256 of the 450 cases (56.8 per cent). One or more of the degenerative lesions or inflammatory nodular lesions were present in 293 of the 450 cases (65.1 per cent). While either inflammatory or degenerative processes may occur alone, there is definite overlapping. An extreme degree of inflammation was more commonly associated with degeneration than a lesser degree. There is a strong suggestion that the inflammation and degeneration may commonly result from the same cause.

COMMENT

An attempt has been made to evaluate the significance of nodular polymyositis and degenerative lesions (atrophy, cytoplastic changes and nuclear variations) in 44 specimens of the deltoid muscle in cases of rheumatoid arthritis, and in seven muscles from each of 450 routine autopsies.

The inflammatory lesions were much more common in the biopsy specimens of the deltoid muscle obtained in cases of rheumatoid arthritis than in the autopsy specimens of the same muscle. This tends to support the belief that this type of muscular lesion is part of the rheumatoid state. The lesions did not differ qualitatively in the two groups. The inflammatory lesions were characterized in the main by lymphocytic infiltration between muscle fibers and muscle bundles. The degenerative lesions were much more common in the cases coming to autopsy, but similar lesions, though fewer, were present in the biopsy material. Whether the nodular areas of myositis represent a characteristic or specific reaction to the possible infective agent of rheumatoid arthritis may be interpreted differently by different observers. Steiner considered the reaction a specific one for rheumatoid arthritis. It is significant that this type of inflammatory reaction was present in all our cases of rheumatic fever. This might be interpreted as suggesting a common relationship between acute rheumatic arthritis and rheumatoid arthritis. In our opinion this type of inflammatory reaction, while decidedly common in rheumatic and rheumatoid arthritis, is not a specific reaction. It was commonly found in muscles in cases of death due to accident or trauma and in many cases in which death was in no way correlated with any type of acute or chronic infection. On the other hand, rheumatoid arthritis to some extent is a common condition in Minnesota and may have been present to some degree without being mentioned in the histories.

There appears to be a definite but not an absolute relationship between the inflammatory lesions and the degenerative processes. They may occur separately, however. The degenerative processes are more commonly associated with a severe inflammation. On the basis of the degree of involvement the inflammation appears probably to be the first and primary lesion. It was noted that inflammation and degeneration were rarely found in the cases of acute poliomyelitis.

With the exception of the cases with rheumatic fever, inflammatory and degenerative types of lesions were more common in older people regardless of the type of disease causing death. The sexes were about equally affected.

Among the large variety of diseases in the autopsy cases there appeared to be no type of disease except acute rheumatic endocarditis in which the inflammatory or the degenerative lesions were more common.

In the single case of acute lupus erythematosus inflammatory nodules were found in six of the seven muscles. There was not a fibrinoid type of reaction in the connective tissue so commonly referred to as a specific reaction in lupus erythematosus.

The impression is gained from the observations in the 44 cases of rheumatoid arthritis and 450 autopsies with multiple causes of death that muscular infection, atrophy, Zenker's degeneration and necrosis are common findings, and while most common in rheumatic and rheumatoid arthritis, these lesions are probably not the result of a specific infectious agent.

CONCLUSIONS

Nodular myositis and muscular degenerative lesions (atrophy, sarcoplastic and nuclear changes) are commonly found in cases of rheumatoid and acute rheumatic arthritis and to a lesser extent in an ordinary series of autopsies.

There appears to be some correlation between the inflammatory and degenerative lesions, but either may be present alone. The degenerative lesions occur more frequently than the inflammatory lesions.

Nodular myositis is more commonly seen in biopsies of deltoid muscles in cases of known rheumatoid arthritis than in postmortem studies of deltoid muscles.

The sexes are about equally involved.

No particular type of disease except rheumatic fever and rheumatoid arthritis seems to increase the frequency of the inflammatory and degenerative lesions.

The lesions are found more frequently in cases in which death occurred in the upper decades of life.

It is doubtful whether the lesions, on a morphologic basis, can be considered as a specific reaction to the infective agent of either acute rheumatic arthritis or rheumatoid arthritis, but the lesions probably are a part of the rheumatic and the rheumatoid state.

A SEARCH FOR CARCINOGENIC SUBSTANCES IN CARCINOMATOUS HUMAN LUNGS

PAUL E. STEINER, M.D., Ph.D.

D. WARREN STANGER, Ph.D.

AND

MIRIAM N. BOLYARD, B.S.

CHICAGO

CARCINOMA of the human lung is unique among the common types of human visceral tumors in that in some cases its causes are known.[1] These causes account for only a small proportion of all pulmonary cancers. A number of agents after being inhaled into the lung are followed by cancer in such a conspicuous number of instances that a cause and effect relationship may reasonably be assumed. These substances may therefore be regarded as exogenous carcinogens. Among these inducers of pulmonic cancers may be mentioned: the ores of mines of Saxony [2] and Bohemia,[3] whose active components may be radioactive substances [4] ; asbestos [5] ; possibly also chromium compounds,[6] iron,[7] nickle [4a] and tar.[8] A critical analysis of this problem is given by Hueper.[4a]

Evidence that additional exogenous carcinogens producing pulmonary carcinoma might exist has come from studies of the occupational incidence of this neoplasm. Thus Kennaway and Kennaway [9] found that in metal

From the Department of Pathology, University of Chicago.

This work was aided by a grant from the Commonwealth Fund.

1. One outstanding exception is the urinary bladder tumor in workers with analine dyes probably induced by beta naphthylamine.

2. Arnstein, A.: Verhandl. d. deutsch. path. Gesellsch. **16**:332, 1913. Härting, F. H., and Hesse, W.: Vrtljschr. f. gerichtl. Med. **30**:296, 1879; **31**:102, 1879.

3. Löwy, J.: Med. Klin. **25**:141, 1929. Pirchan, A., and Sikl, H.: Am. J. Cancer **16**:681, 1932.

4. (*a*) Hueper, W. C.: Occupational Tumors and Allied Diseases, Springfield, Ill., Charles C Thomas, Publisher, 1942. (*b*) Lorenz, E. J.: J. Nat. Cancer Inst. **5**:1, 1944.

5. Holleb, H. B., and Angrist, A.: Am. J. Path. **18**:123, 1942. Lynch, K. M., and Smith, W. A.: Am. J. Cancer **24**:56, 1935.

6. Teleky, L.: Acta, Union internat. contre cancer **3**:253, 1938.

7. Dreyfus, J. R.: Ztschr. f. klin. Med. **130**:256, 1936.

8. Kawahata, K.: Gann **32**:367, 1938.

9. Kennaway, N. M., and Kennaway, E. L.: J. Hyg. **36**:236, 1936.

grinders the incidence of carcinoma of the lung was two and one-fourth times as high as it is in the general population. It was also high in occupations in which workers were exposed to road dust, coal gas and tar, and tobacco. Turner and Grace [10] reported a significantly excessive mortality from cancer of the respiratory system in engineers, foundry workers and grinders.

Pulmonary tumors have been produced with carcinogenic compounds in experimental animals by many methods in numerous laboratories. The methods include direct application of carcinogens to lung tissue,[11] skin painting,[12] oral administration [13] and subcutaneous,[14] intratracheal,[15] intravenous [16] and intraperitoneal injection.[17] Only a few of the methods are here mentioned. The experiments have in common exogenous carcinogens acting on cells of the lungs with resulting tumors.

Atmospheric dusts and substances which are at times found in such dusts have also been tested for their ability to induce tumors. Campbell, in a long series of studies,[18] found a number of dusts and other substances of the environment to be carcinogenic for the lungs of mice. He stated, "There is no fundamental reason why the results obtained in mouse experiments with dusts should not be applied to man." Seelig and Benignus [19] reported that mice exposed to coal smoke soot had an incidence of pulmonary tumors of 8 per cent, compared with 2 per cent in controls. Shimkin and Leiter [20] found a benzene-soluble substance in chimney soot that increased the number of pulmonary tumors in C3H mice. McDonald and Woodhouse [21] caused mice to inhale atmospheric dusts and found that pulmonary tumors developed in 23 per cent, whereas 16 per cent of the controls had tumors. Leiter

10. Turner, H. M., and Grace, H. G.: J. Hyg. **38**:90, 1938.

11. (*a*) Andervont, H. B.: Pub. Health Rep. **52**:1584, 1937. (*b*) Garschin, W. G., and Pigaleff, I. A.: Ztschr. f. Krebsforsch. **33**:631, 1931.

12. Lynch, C. J.: J. Exper. Med. **46**:917, 1927. Murphy, J. B., and Sturm, E.: ibid. **42**:693, 1925.

13. Henshaw, P. S., and Meyer, H. L.: J. Nat. Cancer Inst. **5**:415, 1945.

14. (*a*) Andervont, H. B., and Shimkin, M. B.: J. Nat. Cancer Inst. **1**:225, 1940. (*b*) Heston, W. E.: ibid. **1**:105, 1940. (*c*) Dunlap, C. E., and Warren, S.: Cancer Research **2**:685, 1942. (*d*) Shimkin, M. B.: Arch. Path. **29**:229, 1940. (*e*) Andervont.[11a] (*f*) Dreyfus.[7]

15. Kimura, N.: Japan M. World **3**:45, 1923. Shimkin, M. B.: Am. J. Cancer **35**:538, 1939.

16. Shimkin, M. B.: Arch. Path. **29**:239, 1940. Andervont.[14a]

17. Lynch, C. J.: Proc. Soc. Exper. Biol. & Med. **52**:368, 1943. Henshaw and Meyer.[13]

18. Campbell, J. A.: J. Indust. Hyg. & Toxicol. **19**:449, 1937; Brit. M. J. **1**:179, 1943.

19. Seelig, M. G., and Benignus, E. L.: Am. J. Cancer **28**:96, 1936.

20. Shimkin, M. B., and Leiter, J.: J. Nat. Cancer Inst. **1**:241, 1940.

21. McDonald, S., Jr., and Woodhouse, D. L.: J. Path. & Bact. **54**:1, 1942.

and Shear [22] reported that carcinogenic tars were extractable from air dusts of a number of American cities.

The principle is established that inhaled substances can act as carcinogens in man. It remains to be determined how completely the causation of cancers of human lungs is explained by carcinogens.

An attempt to demonstrate carcinogens in human lungs was reported by Kleinenberg, Neufach and Schabad.[23] They tested benzene extracts from 20 noncancerous persons and 19 cancerous persons, 2 of whom had primary carcinoma of the lungs. The extracts from cancerous persons were injected into 46 mice, which survived to 8 months of age or more. In a mouse which had received an extract of lung from a person who had cancer of the gallbladder, there developed at the site of injection a tumor, diagnosed as sarcoma. The incidence of tumors of other sites was greater in their experimental mice than in the controls. They expressed the belief that they had demonstrated a carcinogen and that the blastomogenic substance was endogenous.

In view of the evidence that certain inhaled substances cause pulmonary carcinoma in man, that additional unrecognized exogenous carcinogens possibly exist and that atmospheric dusts may contain substances that are carcinogenic, at least for animals, it seemed worth while to examine human lungs which contained primary carcinomas as well as control lungs for tumor-inducing substances. Such a study is herewith reported.

SELECTION OF CASES

Experiments were performed with 62 different extracts of 106 lungs from 101 different persons. Sixty of the extracts were made from the lungs of 55 adults; in 5 cases the two lungs were tested separately, because one of each pair contained a primary carcinoma.

These persons had lived in Chicago or the surrounding area. None showed clinical or postmortem evidence of industrial or occupational disease, except that some had excessive amounts of anthracosis. Their degree of exposure to hypothetic atmospheric carcinogens as indicated by their ages in decades was: second, 1; third, 3; fourth, 7; fifth, 6; sixth, 17; seventh, 14; eighth, 6; ninth, 1. Thirty-eight were over 50 years old. Thirty-five were males, and 20 were females. These people probably had been exposed to atmospheric dusts to the degrees average for inhabitants of this community.

The lungs of 46 stillborn infants, because of their small size, were pooled into two specimens (I and II) for extraction and testing. Insufficient material was extracted from the lungs of individual infants to enable a satisfactory biologic test to be performed (average was 0.32 Gm.). Some of these infants showed various stages of prematurity but they had in common the fact that they had not inhaled any air.

The lungs may be divided into five groups:

---

22. Leiter, J., and Shear, M. J.: J. Nat. Cancer Inst. **3**:167, 1942.

23. Kleinenberg, H. E.; Neufach, S. A., and Schabad, L. M.: Cancer Research **1**:853, 1941.

Group A. Lungs Containing Primary Carcinoma.—This group consisted of 12 lungs or pairs of lungs containing a primary cancer.

Group B. Noncancerous Lungs Contralateral to Lungs That Contained Primary Carcinoma.—The nontumorous lung opposite the lung containing a primary carcinoma was examined in 7 cases. If pulmonary cancers are caused by inhaled carcinogens which accumulate slowly and produce tumors only after a relatively long period of induction, the lungs opposite cancerous lungs should also contain the carcinogens, at least in some cases.

Group C. Lungs from Persons Who Had No Cancers.—The 23 lungs composing this group were tested for four reasons: (*a*) They might contain inhaled carcinogens which had not yet induced a tumor. (*b*) They might contain a carcinogen, possibly endogenous, corresponding to that found in human liver.[24b] (*c*) They serve as one type of control for the methods of extraction, yielding information on whether tissue carcinogens are chemical conversion products. (*d*) They add an important organ to those previously studied in a survey of the human body for carcinogens.[25] These persons died from causes commonly encountered in the necropsy service of a general hospital. A list of the diseases represented is given in table 1.

Group D. Lungs from Persons Who Had a Cancer Primary Elsewhere in the Body.—Several of the 18 persons whose lungs composed this group had small pulmonary metastases of tumor, but the majority of the lungs were tumor free. These lungs were tested for the same reasons as were those in group C. In addition, it was desirable to know whether a hypothetic carcinogen transported from a cancer primary elsewhere in the body became localized in the lungs. The types of primary tumors are given in table 1.

Group E. Lungs from Stillborn Infants.—These infants had not inhaled air and their lungs could contain no atmospheric carcinogen. If the extracts showed tumor-inducing activity, the substance would, of necessity, be either an endogenous carcinogen, a chemical conversion product or a carcinogen transmitted from the mother. From other experiments it is known that extracts of the livers of some of these same infants exhibited carcinogenic activity.[25]

PREPARATION OF THE EXTRACTS

The fresh lungs were finely divided with knife or grinder and preserved in 1 volume of 95 per cent ethanol. (Exception: Three lungs were preserved in Kaiserling solution.) Extracts were made by the method previously described.[24a] Essentially it consisted of alkaline hydrolysis and ethylene dichloride extraction of the nonsaponifiable lipids. Saponification was repeated once on the extract so obtained. The final extracts were pale tan or yellow to brown pasty or flaky materials with a pungent odor.

The weight of lung tissue, the amount of extract and the percentage of extract are given in table 1. There were great individual differences in the weights of the lungs because in some cases not all of the lung tissue was available for extraction and because there were great differences in the amount of pathologic involvement present, such as edema, hyperemia, pneumonia and tumor. All the specimens included in group B were single lungs as were the 5 in group A and

24. Steiner, P. E.: (*a*) Cancer Research **2**:425, 1942; (*b*) **3**:385, 1943.

25. Steiner, P. E.; Stanger, D. W., and Bolyard, M. N.: Cancer Research, to be published.

TABLE 1.—*Nonsaponifiable Lipids Extracted from Human Lungs*

Designation of Case, Specimen and Extract	Major Diagnosis	Weight of Lung, Gm.	Weight of Extract, Gm.	Percentage of Nonsaponifiable Lipids
Group A. Lungs containing primary carcinoma:				
5182	Carcinoma of lung	1,953	13.2	0.68
5325	Carcinoma of lung	1,800	9.3	0.52
5456	Carcinoma of lung	436	3.5	0.80
5466	Carcinoma of lung	459	4.0	0.87
5470	Carcinoma of lung	1,811	5.1	0.28
5506	Carcinoma of lung	925	5.6	0.57
5533	Carcinoma of lung	873	4.4	0.50
5550	Carcinoma of lung	968	5.0	0.52
5564	Carcinoma of lung	810	5.7	0.70
5627	Carcinoma of lung	867	3.4	0.39
5682	Carcinoma of lung	744	2.4	0.32
5685	Carcinoma of lung	821	2.7	0.33
Group B. Noncancerous lungs contralateral to lungs that contained primary carcinoma:				
5355	Carcinoma of lung	291	1.6	0.55
5458	Carcinoma of lung	309	3.4	1.10
5466	Carcinoma of lung	341	2.5	0.73
5550	Carcinoma of lung	285	2.3	0.81
5564*	Carcinoma of lung	563	1.7	0.30
5575	Carcinoma of lung	480	2.4	0.50
5682	Carcinoma of lung	629	2.2	0.35
Group C. Lungs from noncancerous persons:				
5368	Pulmonary tuberculosis	1,130	3.3	0.29
5519	Malignant nephrosclerosis	750	5.6	0.75
5521	Pyelonephritis and diabetes	327	2.2	0.67
5522	Coronary thrombosis	730	3.8	0.52
5525	Nonspecific ulcerative colitis	528	1.5	0.28
5527	Nonspecific ulcerative colitis	783	2.8	0.36
5528	Abscess of the lung	638	2.8	0.31
5531	Streptococcic pyemia	1,014	3.8	0.37
5542	Fracture of femur	355	5.6	1.58
5549	Rheumatic heart disease	750	2.7	0.36
5581	Hypertensive cardiovascular disease.	936	5.8	0.62
5603	Rheumatic heart disease	435	1.9	0.44
5607	Streptococcic septicemia	275	1.2	0.44
5610	Rheumatic heart disease	387	2.1	0.54
5623	Pulmonary embolism	775	3.0	0.39
5636	Bile peritonitis	625	2.4	0.38
5638	Arteriosclerotic heart disease	1,230	3.7	0.30
5639	Bacterial endocarditis	360	1.3	0.36
5644	Glaucoma	472	1.5	0.32
5645*	Rapidly progressive hypertension	1,080	5.9	0.55
5648	Encephalomyelitis	820	2.2	0.27
5657	Malignant nephrosclerosis	860	5.6	0.65
5661	Congenital renal agenesis	345	1.6	0.46
Group D. Lungs from persons with nonpulmonic cancers:				
5544	Carcinoma of stomach	395	2.1	0.53
5571	Carcinoma of stomach	1,780	6.9	0.39
5599	Carcinoma of stomach	341	1.3	0.38
5655	Carcinoma of stomach	1,170	3.7	0.32
5578	Leukemia	620	3.0	0.48
5612	Leukemia	790	4.4	0.56
5617	Leukemia	840	2.9	0.34
5541	Carcinoma of colon	732	1.8	0.25
5614	Carcinoma of colon	424	2.3	0.54
Eng. 41	Carcinoma of kidney	1,215	8.7	0.72
5532	Carcinoma of kidney	751	3.3	0.44
5667*	Carcinoma of prostate	795	2.6	0.33
5680	Carcinoma of prostate	793	2.0	0.25
5529	Carcinoma of breast	308	1.6	0.52
5569	Carcinoma of uterus	990	3.4	0.34
5653	Carcinoma of liver	1,180	5.0	0.42
5658	Carcinoma of bile ducts	1,150	3.8	0.33
5656	Lymphoblastoma	920	3.9	0.42
Group E. Lungs from stillborn infants:				
I	(26 pairs of lungs)	1,006	6.7	0.67
II*	(20 pairs of lungs)	1,049	8.0	0.76

* These extracts later proved to be carcinogenic.

scattered specimens in the other groups. The extracts also varied greatly in amount because they represented the nonsaponifiable lipids not only of the original pulmonic tissue but also of any tumor, inflammatory exudate and other solid component which was present. The extracts expressed in percentage of weight of original tissue also show great variation, partly at least because there were great differences in the amount of pulmonary edema and other fluids. These factors combine to hide any possible differences in the amount of extract attributable to inhalation of carcinogenic materials. This statement is true regardless of whether the comparison is by individual cases (table 1) or by groups (table 2).

The extracts were mixed with 3 volumes of tricaprylin for injection.

TESTING THE EXTRACTS

The extracts were tested for carcinogenic activity as follows: Mice were used. Each animal was given by subcutaneous injection a total of about 500 mg. in 1.5 cc. of tricaprylin. This amount was administered in two doses given four weeks apart. The number of mice receiving injections in each experiment varied from 3 to 27, according to the amount of extract available. The mice were about

TABLE 2.—*Summary of Extractions of Nonsaponifiable Lipids of Human Lungs*

Experimental Group	Persons from Whom Lungs Were Obtained	Average Weight of Lungs, Gm.	Average Weight of Extract, Gm.	Percentage of Nonsaponifiable Lipid Extract
A	12	1038.9	5.36	0.52
B	7	414.0	2.30	0.56
C	23	678.4	3.14	0.47
D	18	844.1	3.48	0.41
E	46	44.6	0.32	0.71

equally divided as to sex. They were of two strains. Thirty-seven extracts were tested in 315 mice of the C57 Black strain. These mice were from 2½ to 6½ months old. Some were obtained from the Roscoe B. Jackson Memorial Laboratory and some were raised in our laboratory as the first generation offspring of breeding stock obtained from Bar Harbor. Twenty-five extracts were injected into 129 mice of our own partly inbred albino strain.[26] These mice were from 2 to 5 months old.

The extracts of infant lungs were toxic in doses of 250 mg. dispersed in 0.75 cc. of tricaprylin. Consequently they were administered at the rate of 125 mg. in 0.37 cc. of tricaprylin at intervals of four weeks. The mice used for the group E I extract received a total of only 375 mg. of extract, but those used for the group E II extract had the usual 500 mg.

The mice were fed a diet previously described.[24b] They were examined at frequent intervals. Necropsies were performed on all animals, and all lesions suggestive of neoplasm were examined microscopically. The experiments were terminated early in the twenty-fifth month.

Control experiments were performed in which 154 mice of the C57 Black strain were given by subcutaneous injection 2 cc. of tricaprylin alone. This control was made because the diet and possibly other environmental conditions of this laboratory are different from those at Bar Harbor.

26. Steiner and others.[25] Steiner.[24]

RESULTS

One lung extract (5525), obtained in a case of nonspecific ulcerative colitis, exhibited immediately after injection the high toxicity previously observed in cases of this disease [27] and noted occasionally to a lesser degree with other human tissue extracts. [26] It killed all mice, and consequently it was not tested for carcinogenicity. The extracts of both the pooled specimens of infant lungs also were toxic, but tests were successfully made by injecting them in four doses.

Some extracts were caustic to the tissues and sloughed out wholly or in part. This phenomenon has previously been observed with extracts of human liver, spleen, colon and other materials.[26] It is again mentioned because it prevented quantitative determinations of carcinogenic potency. This necrotizing factor was not recognizably related to the major disease diagnosed or to the subsequent carcinogenicity of the extract. The estimated losses of the extract in percentage were as follows: 0, 3 cases; 1 to 24, 23 cases; 25 to 49, 14 cases; 50 to 74, 12 cases; 75 to 100, 9 cases.

Survival of the mice was satisfactory in most experiments. Of the 474 originally receiving injections, 413 survived for six months, 355 for twelve months, 220 for eighteen months, and 74 were alive at the beginning of the twenty-fifth month after injection, when the experiments were terminated.

Six sarcomas were found at sites of injections of four extracts, of which three were from adult lungs and one from lungs of infants. The cases are abstracted as follows:

5564. The nontumorous lung from which the extract was obtained and which was the mate of a lung containing primary carcinoma was that of a railroad brakeman 56 years old. It weighed 563 Gm. and yielded 1.7 Gm. of extract, which was injected into 4 C57 Black male mice. One mouse died in the thirteenth month with a large mixed spindle and polymorphous cell sarcoma at the site of injection. Another mouse died in the eighteenth month with a similar tumor. One died in the tenth month with a reticulum cell sarcoma involving the liver, the spleen and the lymph nodes. The last animal died in the twenty-third month without tumor.

5645. The extract (5.9 Gm.) was obtained from the lungs of a business man 56 years old who died of rapidly progressive hypertension. It was tested in 12 albino strain male mice. One died in the eighteenth month of a large spindle cell carcinoma of the injection site, leaving 4 survivors, 2 of which subsequently succumbed to pulmonary tumors in the twenty-first and twenty-fourth months.

5667. The extract (2.6 Gm.) was obtained from the lungs of a decorator aged 62 years who had a carcinoma of the prostate. It was tested in 5 albino strain male mice. One spindle cell sarcoma was found at the site of injection in the twenty-fourth month. At the same time 2 mice had pulmonary tumors.

27. Steiner, P. E.; Stanger, D. W., and Bolyard, M.: Proc. Soc. Exper. Biol. & Med. **55**:8, 1944.

Stillborn Infant Pooled Speciment II. The extract (8.0 Gm.) was injected into 16 albino strain female mice, of which 11 were alive at twelve months, 8 at eighteen months and 1 at twenty-four months after injection. Mice died with spindle cell sarcomas at the injection sites in the twenty-first and twenty-fourth months. In addition, 4 animals died of leukemic disease in the eighteenth, twenty-first, twenty-second and twenty-third months; 1, of pulmonary tumor in the seventeenth month, and 1, of mammary gland carcinoma in the twenty-fourth month.

In table 3 the results are summarized with respect to the sarcomas at sites of injection. The percentage yield (calculated on the basis of the number of mice living at the time when the first sarcoma appeared in each experiment) varied from 20.0 to 66.7, with an average of 33.3.

TABLE 3.—*Sarcomagenic Activity of Nonsaponifiable Lipids Extracted from Human Lungs*

Experimental Group	Source of Extracts	Extracts Tested	Mice Used	Mice Alive at 6 Mo.	Sarcomagenic (Active) Extracts	Effective Total* Number of Mice in Which Extracts Were Active	Sarcomas Induced	Percentage Yield with Active Extracts
A	Lungs with primary carcinoma.....	12	129	115	0	0	0	0
B	Noncancerous lungs contralateral to lungs with primary carcinoma.....	7	33	31	1	3	2	66.7
C	Lungs from noncancerous persons..	23	146	131	1	5	1	20.0
D	Lungs from persons with cancer other than pulmonic.....	18	127	119	1	3	1	33.3
E	Lungs of stillborn infants (pooled)..	2	39	12	1	7	2	29.9

* The number of mice living when the first tumor appeared is used as the "effective total" number.

Summaries of experiments in which tumors occurred or did not occur are given in table 4, together with other pertinent data. The experiments are divided into three sections: (*a*) those experiments in which sarcomas were found at sites of injection, (*b*) those in which no sarcomas occurred and (*c*) tricaprylin controls. This arrangement is used to facilitate the analysis of tumors which occurred at distant sites.

The subcutaneous spindle and polymorphous cell sarcomas which were found at sites of injection can safely be regarded as induced tumors inasmuch as such neoplasms do not develop spontaneously in either of the strains of mice used. Whether they were induced by a sarcogenic component of the extracts might be challenged, because 1 control mouse, given tricaprylin alone, had a similar tumor. The differences in the percentage yield of sarcomas (calculated on the basis of the number of tumors in the mice surviving the injection six months) are, however, probably significant in view of the number of mice tested (18.8 per cent versus 0.7 per cent). The extracts are regarded as having shown carcinogenic activity.

TABLE 4.—*Summaries of Experiments in Which Tumors Were or Were Not Produced with Extracts of Human Lungs*

Designation of Case, Specimen and Extract	Mice Receiving Injections			Mice Surviving Given Number of Months				Sarcomas at Sites of Injection		All Other Tumors							
										Lung		Lymphatic		Mammary Gland		Miscellaneous	
	Strain	Sex	No.	6 Mo.	12 Mo.	18 Mo.	24 Mo.	No.	%*	No.	%	No.	%	No.	%	No.	%
Experiments in which sarcomas developed at sites of injection:																	
5564 (noncancerous)	C57 Black	Male	4	4	3	1	0	2	50.0	0	0.0	1	25.0	0	0.0	0	0.0
5645	Albino	Male	12	12	10	4	0	1	8.3	2	16.6	0	0.0	0	0.0	0	0.0
5667	Albino	Male	5	5	5	4	3	1	20.0	2	40.0	0	0.0	0	0.0	0	0.0
Infant lungs, II	Albino	Female	16	11	11	8	1	2	18.2	1	9.1	4	36.4	1	9.1	0	0.0
Totals	.....	....	37	32	29	17	4	6	18.8	5	15.6	5	15.6	1	3.0	0	0.0
Experiments in which no sarcomas developed at sites of injection:																	
36 combined cases	C57 Black	Both	311	265	246	169	62	0	0.0	6	2.2	32	12.1	0	0.0	10	3.8
22 combined cases	Albino	Both	126	116	96	46	12	0	0.0	18	15.5	4	3.5	4	3.5	1	0.9
Totals (58 cases)	.....	....	437	381	342	215	74	0	0.0	24	6.3	36	9.6	4	1.1	11	3.0
Tricaprylin controls:																	
.....	C57 Black	Both	154	149	116	72	0	1	0.7	2	1.4	10	6.7	0	0.0	0	0.0

* In this table the percentage yield is calculated from the number of tumors in six month survivors.

The tumors found elsewhere in the mice—the lungs, the lymphatic system, the mammary glands and other structures—are also given in table 4. Tumors of distant sites can be induced by subcutaneous injections of carcinogens. If the incidence of such tumors is significantly increased above normal for the strain by the injection of substances of unknown carcinogenic activity, it may be concluded that the material is carcinogenic. In the present analysis the incidence of tumors of the lungs appears greater in the combined experiments in which the extracts were locally sarcomagenic than in those in which they were not, or in the tricaprylin controls. However, most of the increase was contributed by albino strain mice. When the figures for the incidence of pulmonary tumors in the two groups of experiments are compared for each strain separately, no increase is found. A small increase in lymphatic tumors (12.1 versus 6.7 per cent) was found in C57 Black mice given injections of lung extracts when these mice were compared with trycaprylin controls. This constitutes the only acceptable evidence for an increase of tumors of distant sites.

COMMENT

Evidence that a carcinogenic factor was present in extracts of some human lungs was obtained in these experiments. The extracts induced sarcomas at sites of their injection, and they probably increased the incidence of lymphatic tumors in C57 Black mice

Evidence was also obtained that the active factor was endogenous rather than exogenous; it was already present in the lungs of stillborn infants who had not inhaled air, although some of them probably had aspirated amniotic fluid. Transplacental transmission of an exogenous factor was not eliminated as a possibility, but it seems highly remote because of the failure to detect activity in the majority of extracts of adult lungs In other experiments extracts of the livers of some of these same infants were carcinogenic, pointing again to an endogenous factor.

Failure to demonstrate exogenous carcinogens in these experiments does not prove their absence. Only one method of extraction was used, and only one type of extract was tested. Furthermore, it should be noted that none of the carcinomas of the lungs which were tested had been induced by any of the recognized or strongly suspected exogenous pulmonary carcinogens. Neither did the lungs exhibit any of the industrial or occupational diseases which sometimes accompany or terminate in cancer. Studies of such lungs by this and other methods might prove profitable.

These experiments revealed no causative exogenous factor for pulmonary cancer. The three extracts of adult lungs which were sarcomagenic came from the control groups. One extract (5564) which

induced 2 tumors in 4 mice was made from a noncancerous lung whose mate contained a primary carcinoma and yielded an inactive extract. The 3 adult lungs which gave sarcomagenic extracts did not have any pulmonic disease in common. Two factors were shared by all 3: (*a*) They were all from men. (*b*) These men all had disease of the prostate; one had primary carcinoma of the prostate gland, and the others had benign adenomatous hyperplasia despite their age, 56 years, which is below the usual age for this condition. The possible relationship of sarcomagenic activity of human tissue extracts and hormonal imbalances has previously been mentioned.[24b] Its significance, if any, in the present instance is not known.

Human lung may be added to liver and spleen as examples of tissues which in a survey of the body have yielded sarcomagenic extracts.[25]

It is known from studies of carcinogens that minute quantities may induce tumors in animals. Shear [28] reported that 0.0004 mg. of 1, 2, 5, 6-dibenzanthracene induced a sarcoma in a mouse. It is possible that small quantities of a carcinogen may induce tumors in man and that in the process the chemical is changed to a new form. It might be difficult or impossible to recover the carcinogen from such tumors. For these reasons failure to demonstrate carcinogens does not prove their absence. If a carcinogen were obtained from a cancerous lung, it might represent merely an excess, an unutilized residue, and not that portion which actually induced the tumor.

Four of 62 extracts that were tested induced sarcomas. This result is not to be interpreted as an absolute and final measure of the degree of carcinogenicity of lung extracts, any more than were the results of testing extracts of individual livers.[24b] There appears to be a definite "threshold" phenomenon in the testing of these crude tissue extracts. It is probable that both the number of extracts which induced tumors and the number of tumors which they induced would have been increased if any or all of the following conditions had prevailed: (*a*) If after injection the material had been better retained. (*b*) If the mice had lived longer. (*c*) If mice more highly susceptible to sarcoma had been used. The results obtained pertain only to the experiments performed. Better methods may give different results in the future.

SUMMARY

The nonsaponifiable lipid extracts of 106 human lungs were tested for carcinogenic activity in 62 separate experiments in which 474 mice of C 57 Black and our albino strains were used. The lungs were those containing primary carcinoma; noncancerous lungs contralateral to those containing primary carcinoma; lungs of persons free from cancers;

28. Shear, M. J.: Am. J. Cancer **26**:322, 1936.

lungs of persons with cancer primary elsewhere in the body, and lungs of stillborn infants.

Six sarcomas were induced at sites of injection of four different extracts. This constitutes a 33.3 per cent yield if the yield is calculated on the basis of the number of mice living when the first tumor appeared in each of the experiments with active extracts. The percentage yield calculated on the basis of the number of tumors in six month survivors was 18.8. In addition, the incidence of lymphatic tumors in C 57 Black mice was possibly increased above normal.

The sarcomagenic extracts were derived, respectively, from a nontumorous lung opposite a lung with primary carcinoma, the noncancerous lungs of a person with carcinoma of the prostate, the lungs of a person with rapidly progressing hypertension, and the pooled lungs of stillborn infants.

The sarcoma-inducing activity of an extract of lungs of infants who had not inhaled air indicates that the sarcomagen is probably endogenous. Human lung may be added to liver and spleen as examples of tissues from which extracts with tumor-inducing activity have been obtained.

NONSPECIFIC MYOCARDITIS

Analysis of a Series of Thirty-Six Cases

PETER M. MARCUSE, M.D.
HOUSTON, TEX.

THE PROBLEM of myocarditis has received increasing attention in recent years. Most of this interest has been confined to the isolated form which is also known as Fiedler's myocarditis. The importance of this disease is illustrated by the fact that Moritz and Zamcheck [1] found 14 cases of isolated myocarditis among "between 200 and 300" instances of unexpected death in young soldiers.

In most of the reported cases of Fiedler's myocarditis the leukocytic infiltration was of a diffuse type. There is, however, evidence suggesting that the anatomically less conspicuous focal type of inflammation may also be of considerable importance. Saphir,[2] reporting 5 cases of myocarditis combined with laryngeal edema in children, stated that the areas of inflammation were widely scattered throughout the myocardium and that in 2 instances ten blocks had to be sectioned before such lesions were found. Clinical observations further add to the concept that focal myocarditis is not infrequent and that it may be of practical import. Glatthaar [3] expressed the belief that foci of inflammation around vital branches of the coronary arteries or within the conductive system may be responsible for cardiac symptoms as well as for electrocardiographic changes and occasionally also for sudden death. Scherf [4] found that clinical evidence of myocardial involvement developed in 10 to 15 per cent of patients with acute tonsillitis. Scherf and Boyd [5] go as far as to imply that in view of the frequency of infectious diseases and localized infections there are but few persons who at some time or another do not have small inflammatory myocardial foci. Candel and Wheelock [6] arrived at a similar opinion from their observations in 11 cases of acute nonspecific myocarditis.

From the Department of Pathology, Baylor University College of Medicine.

1. Moritz, A. R., and Zamcheck, N.: Arch. Path. **42**:459, 1946.
2. Saphir, O.: Am. J. M. Sc. **210**:296, 1945.
3. Glatthaar, D.: Schweiz. med. Wchnschr. **76**:74, 1946.
4. Scherf, D.: Bull. New York M. Coll., Flower & Fifth Ave. Hosp. **3**: 252, 1940.
5. Scherf, D., and Boyd, L. J.: Cardiovascular Diseases, St. Louis, C. V. Mosby Company, 1939, pp. 176-180.
6. Candel, S., and Wheelock, M. C.: Ann. Int. Med. **23**:309, 1945.

It appears therefore justified to include instances of focal myocarditis in the series presented here.

SELECTION OF CASES

The group comprises 36 cases that were collected from a total of 3,800 autopsies. The diagnosis was based on the microscopic examination of routine sections. In the large majority of cases the blocks were taken from the wall of the left ventricle or from the interventricular septum. On the average, two blocks were cut in each case. In general, only those instances were included in which there was either extensive diffuse leukocytic infiltration or unequivocal evidence of focal inflammation. However, for reasons explained later, 4 cases with minimal focal inflammatory involvement were added. Cases in which any of the following findings were made at autopsy were not included in this series: rheumatic heart disease, myocardial changes due to lesions of the coronary arteries, extensive myocardial fibrosis, bacterial endocarditis, acute pericarditis, pyemic abscesses of the myocardium and specific granulomas. Also excluded were all cases of specific infections that are known to cause myocardial lesions, such as diphtheria and scarlet fever.

It is obvious that difficulties could arise in ruling out rheumatic lesions. All cases were excluded in which a history of rheumatic fever was given or in which Aschoff bodies, epicardial or endocardial involvement unless of a minimal degree, fibrinoid swelling or perivascular fibrosis was shown. Syphilis as a cause was eliminated as far as possible by omitting all cases in which a history of syphilitic infection was given or in which serologic tests were positive, as well as those in which the autopsy findings were indicative of syphilis. Results of serologic tests were available in only about one half of the cases, mainly because many of the patients died after having been in the hospital for only a short time.

Cases of chronic myocarditis with formation of giant cells and granulation tissue, such as the case of "myocarditis perniciosa" described by Boikan,[7] were not added to this series since it appeared hardly possible to differentiate the lesions from specific granulomas.

PRESENTATION OF FINDINGS

Age.—The ages of the patients ranged from 1 month to 86 years. Twenty-two of the patients were below the age of 40, and 8 of these were less than 15 years old.

Sex.—The group included 26 males and 10 females. The percentage of males in the entire autopsy series was 65.

7. Boikan, W. S.: Virchows Arch. f. path. Anat. **282**:46, 1931.

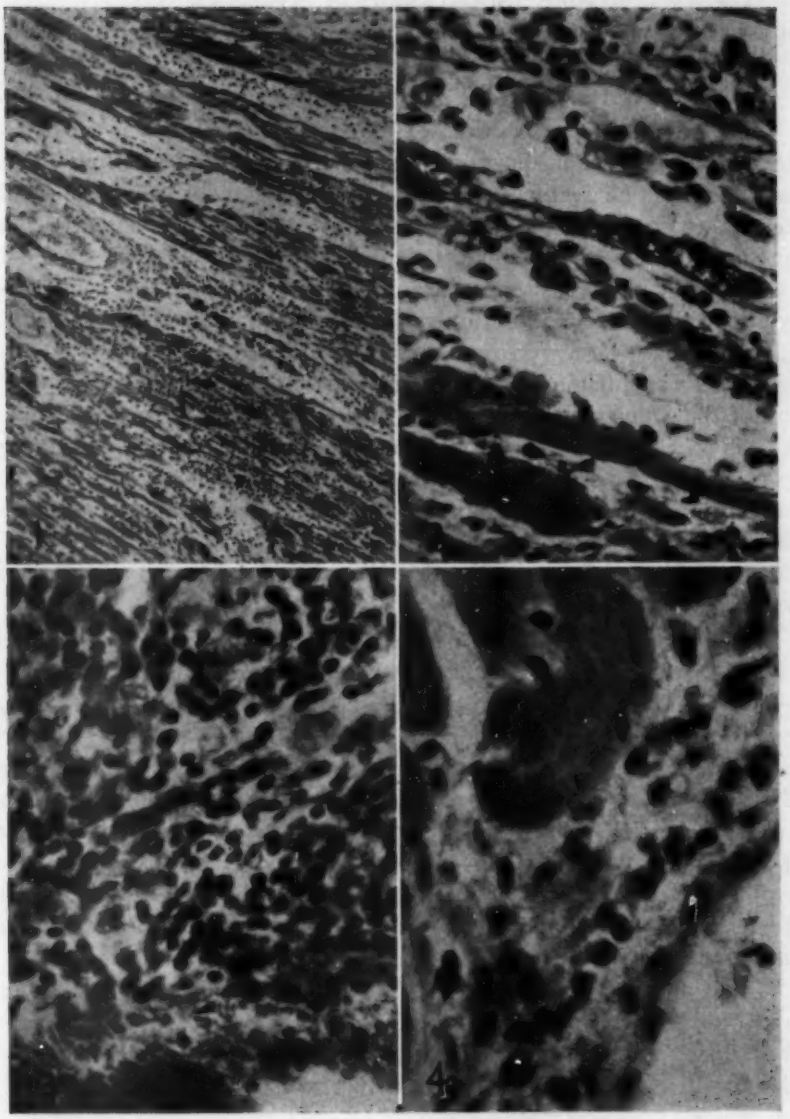


Fig. 1.—Diffuse myocarditis; × 65. The infiltration is most prominent in the interstitial tissue.

Fig. 2.—Diffuse myocarditis; × 250. Infiltration of an interstitial space is shown, with involvement of the adjacent muscle fibers.

Fig. 3.—Focal myocarditis; × 250. A large focus is destroying muscle fibers immediately adjacent to an interstitial blood vessel.

Fig. 4.—Focal myocarditis; × 450. This is a perivascular lesion containing elongated cells with distorted nuclei.

Gross Findings.—These were, in general, confined to cardiac hypertrophy, which was noted in 15 cases. Only in a few instances a gray discoloration of the myocardium was seen. In over half of the cases gross changes were absent.

Microscopic Changes.—The basic lesion was a leukocytic infiltration, which was always of interstitial nature, frequently showing a tendency toward perivascular arrangement (fig. 1). In many cases there were, in addition, infiltration and partial destruction of some of the myocardial fibers. The parenchymal changes seemed to be of a secondary nature since, with rare exceptions, the lesions in the fibers could be traced to interstitial infiltrates in adjacent areas (figs. 2 and 3).

The cellular composition is analyzed in table 1. The only constant finding was the presence of elongated cells with large, distorted nuclei. Although cells of this type were never predominant, they were found to be present in most instances.

TABLE 1.—*Distribution and Composition of Myocardial Infiltrates*

	Cases in Which the Predominant Cells Were			Cases in Which No Type of Cell was Predominant	Cases in Which Eosinophils Were Present
Distribution	Polymorphonuclear Leukocytes	Plasma Cells	Lymphocytes		
Diffuse.....	5	4	3	..	..
Focal.....	10	2	8	..	3
Focal and minimal	..	..	..	4	..

The type cell has a compact, dark-staining nucleus, which is often curved or distorted and which averages 10 microns in length. The cytoplasm is scanty and indistinctly outlined, is faintly acidophilic and forms short tapering processes at the poles of the cell (figs. 4 and 5).

These cells are morphologically different from the "myocyte" of Anitschkow and from the Aschoff cell. Their distribution precludes an origin from degenerating muscle fibers and makes it likely that they represent proliferating fibroblasts or endothelial cells. They appear to be related to the mesenchymal cells that are seen in rheumatic lesions and that might be the forerunners of the multinucleated giant cells.

Extracardiac Foci of Inflammation.—Inflammatory lesions in organs other than the heart could be found in 35 of the 36 cases. The distribution of the foci, as well as the character of the pulmonary lesions, are given in table 2. The extrapulmonary lesions were distributed among a wide variety of organs or structures (skin, liver, kidneys, peritoneum, prostate, gallbladder, brain and meninges).

Clinical Evidence of Cardiac Involvement.—Objective signs were present in 9 cases. Tachycardia was noted in 5 instances, cardiac enlargement in 2, arrhythmia in 1 and murmurs in 1.

Evidence of Drug Sensitization.—The application of sulfonamide compounds was recorded in 6 cases and could be definitely ruled out in 17 others. In the remaining 13 cases the role played by sulfonamide drugs could not be determined with certainty, especially since some of the patients had had treatment before entering the hospital. Only 1 instance of treatment with neoarsphenamine was found.

COMMENT

In this series nonspecific myocarditis was found in about 1 per cent of autopsies. Since the lesions may be small and widely separated, it appears likely that they remained unnoticed in some instances and that the true incidence is, therefore, higher than 1 per cent.

Considerable difficulty was encountered in deciding which of the cases should be designated as instances of "isolated" myocarditis. According to Saphir[8]:

> . . . one is justified in accepting the occurrence of isolated myocarditis in the sense of a more or less diffuse inflammatory lesion if every known cause for this type of myocarditis is ruled out and if the myocarditis is found in the absence of any major pathologic condition involving either the endocardium and pericardium or the entire body.

TABLE 2.—*Site and Classification of Extracardiac Lesions*

Site	Cases
Lungs only	18
Lungs and other organs	8
Other organs only	9
Classification of Pulmonary Lesions	
Bronchopneumonia	8
Interstitial pneumonitis	8
Tuberculosis	4
Septic infarcts	1
Pleuritis	2
Lipid pneumonia	1
Bronchiectasis	2

In none of the cases presented here was any endocardial or pericardial involvement shown; but, with regard to lesions of other organs, it was difficult to decide which of these had to be called major pathologic conditions. Ten of the 12 cases with diffuse leukocytic infiltration of the myocardium should meet the requirements established for the isolated type. In 9 of them extracardiac foci were demonstrated only in the lungs, and none of these lesions were extensive; in the tenth there was evidence of chronic prostatitis.

It appears doubtful, however, whether a sharp line can be drawn between focal and diffuse involvement as well as between isolated and

8. Saphir, O.: Arch. Path. **32**:1000, 1941.

complicating myocarditis. It seems more likely that the diffuse inflammation is merely an advanced stage due to confluence of disseminated lesions and that, as in the other types of myocarditis, inflammatory foci are found elsewhere in the body.

The question whether or not myocarditis is primarily of interstitial nature has been the occasion of considerable argument. Some authors present evidence that the process begins with damage of the myocardial fibers (Hansmann and Schenken [9]; Covey [10]). In the series of cases reported here, the inflammatory process seemed to have originated in the interstitial connective tissue, and any involvement of the muscle fibers was apparently of secondary nature. This impression was enhanced by examination of the group classified as "minimal myocarditis," in which the lesions were found exclusively within the

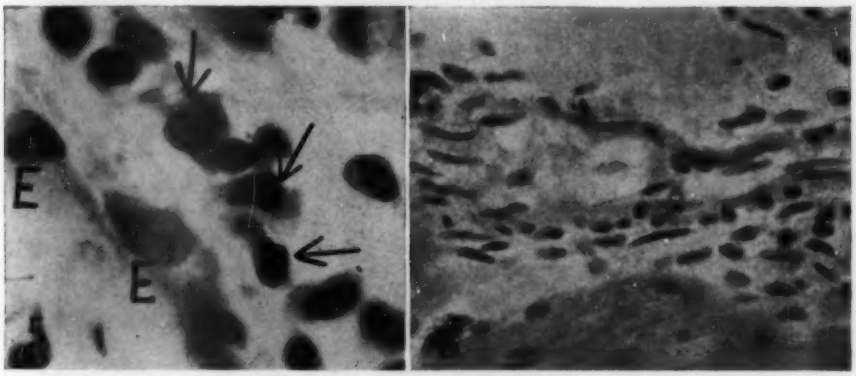


Fig. 5.—Part of the area shown in figure 4; × 950. Arrows point to typical elongated cells with distorted nuclei. Cells marked *E* belong to the endothelium of a capillary.

Fig. 6.—Focal myocarditis; × 455. This is a minimal focus in close vicinity to a capillary.

fibrous tissue septums. The 4 cases of minimal myocarditis were included in this series since, although the foci were minute, the same characteristic histologic picture was shown in all of them. The lesions were confined to the interstitial tissue, the myocardial fibers being intact in all 4 cases. The cellular elements consisted of small round cells, plasma cells and mesenchymal cells with distorted nuclei. Occasionally a small number of polymorphonuclear leukocytes were present. Increased vascularization was constantly found in the form of dilated thin-walled capillaries. In many instances the lesions were located close to interstitial arterioles.

9. Hansmann, G. H., and Schenken, J. R.: Am. Heart J. **15**:749, 1938.

10. Covey, G. W.: Am. J. Clin. Path. **12**:160, 1942.

Since minimal foci were occasionally found in the other cases, together with more extensive areas of inflammation (fig. 6), it appears likely that in the 4 instances of minimal myocarditis the process represents early stages of more severe involvement.

Although no cases were included in which any of the accepted anatomic stigmas of rheumatic disease were shown, there is no way to

TABLE 3.—*Review of Possible Causes*

Cause	Author	Nature of Evidence: Clinical	Post-mortem	Experimental
Bacterial infections	Scherf [4]	+	..	..
	Schenken and Heibner [20]	..	+	..
	Glatthaar [3]	+	..	..
Virus	Pearce, J. M.: Arch. Path. **34**: 319, 1942	..	..	+
	Covey [13]	+	+	..
	Finland and co-workers [14]	..	+	..
	Helwig, F. C., and Schmidt, E. C. H.: Science **102**: 31, 1945	..	..	+
Sensitization to various substances	Apitz, K.: Virchows Arch. f. path. Anat. **289**: 46, 1933	..	..	+
	Clark E., and Kaplan, B. I.: Arch. Path. **24**: 456, 1937	..	+	..
	Brown, C. E., and McNamara, D. H.: Arch. Dermat. & Syph. **42**: 312, 1940	..	+	..
	Rich, A. R.: Bull. Johns Hopkins Hosp. **71**: 123, 1942	..	+	..
	French, A. J., and Weller, C. V.: Am. J. Path. **18**: 109, 1942	..	+	+
	Chafee, F. H.; Ross, J. R., and Gunn, E. M.: Ann. Int. Med. **17**: 45, 1942	..	+	..
Drugs causing increased heart action	Fleischer, M. S., and Loeb, L.: Arch. Int. Med. **6**: 427, 1910	..	..	+
	Hueper, W. C., and Ichniowski, C. T.: J. Lab. & Clin. Med. **26**: 1565, 1941	..	..	+
Dietary deficiency				
General malnutrition	Toreson, W. E.: Arch. Int. Med. **73**: 375, 1944	..	+	..
Potassium deficiency	Schrader, G. A.; Prickett, C. O., and Salmon, W. D.: J. Nutrition **14**: 85, 1937	..	..	+
	Follis, R. H.; Orent-Keiles, E., and McCollum, E. V.: Am. J. Path. **18**: 29, 1942	..	..	+
Combined deficiency of potassium and vitamin B	Thomas, R. M.; Mylon, E., and Winternitz, M. C.: Yale J. Biol. & Med. **12**: 345, 1940	..	..	+

rule out a rheumatic process with absolute certainty. The leukocytic collections that always accompany the Aschoff bodies may in exaggerated cases appear in the form of a heavy diffuse infiltration (Sacks [11]). Von Glahn [12] described masses of distorted cells and stated that he considered these, together with polymorphonuclear leukocytes, to be as distinctive as the Aschoff bodies. Since elongated and distorted cells were frequently found in the series reported here, the question arises whether there might be a connection between nonspecific myo-

11. Sacks, B.: Am. Heart J. **1**:750, 1926.

12. von Glahn, W. C.: Am. J. Path. **2**:1, 1926.

carditis and rheumatic myocarditis. It appears possible that both constitute a response of the interstitial tissue to an inflammatory focus elsewhere in the body.

In addition to the involvement of the heart there were in practically all cases lesions of other organs, particularly of the lungs. As bronchopneumonia is often a terminal event the importance of this lesion as a focus of inflammation may be subject to doubt. Since, however, bronchopneumonia alone was present in only 6 cases, this criticism could not materially subtract from the impression that myocarditis is generally connected with other inflammatory processes. Pulmonary lesions are mentioned in only few reports in the literature (Saphir [13]; Finland and others [14]; Covey [15]), and Saphir [13] stated that inflammatory disease of the lungs is not commonly associated with myocarditis.

Much has been written about causation of nonspecific myocarditis, and the possible causes mentioned in the literature are briefly reviewed

TABLE 4.—*Results of Bacteriologic Studies Made During Life*

	Cases in Which No Bacteria Were Demonstrated	Cases in Which Given Bacterium Was Shown		
		Staphylococcus	Pneumococcus	Mycobacterium Tuberculosis
Smears from various sources (throat, skin, sputum)	1	3	3	3
Blood cultures	2	2	1	

in table 3. In addition, myocarditis arising in the course of pregnancy has been described (Gouley and associates [16]) and in uremia (Solomon and associates [17]). None of these possibilities have so far proved to be convincing. The main difficulty of an experimental approach lies in the fact that myocarditis occurs spontaneously with considerable frequency in experimental animals (Miller [18]). In the series under discussion the only constant finding was that of accompanying inflammatory lesions of other organs. The elevation of the leukocyte count observed in most cases points to the presence of an active inflammatory process (table 4).

The effect of sulfonamide compounds could be definitely ruled out in 12 cases. The fact that eosinophils were found in noteworthy numbers

13. Saphir, O.: Arch. Int. Med. **72**:775, 1943.

14. Finland, M.; Parker, F.; Barnes, M. W., and Joliffe, L. S.: Am. J. M. Sc. **209**:455, 1945.

15. Covey, G. W.: Am. J. Clin. Path **12**:160, 1942.

16. Gouley, A. B.; McMillan, T. M., and Bellet, S.: Am. J. M. Sc. **194**:185, 1937.

17. Solomon, C.; Roberts, J. E., and Lisa, J. R.: Am. J. Path. **18**:729, 1942.

18. Miller, C. P.: J. Exper. Med. **40**:543, 1924.

in only 3 cases further speaks against the presence of a sensitizing agent. There was no evidence that syphilis played a causal role.

The paucity of clinical evidence is illustrated by reports of sudden death in apparently healthy persons (Moritz and Zamcheck [1]; Didion [19]; Saphir [2]). In the group of cases reported here the clinical manifestations of cardiac involvement were scanty, and in no instance was even a tentative diagnosis of myocarditis made.

With few exceptions (Schenken and Heibner [20]) the reports in the literature do not include bacteriologic studies. In several of the cases in the series presented here various types of bacteriologic tests were performed during life, and the results are given in table 5. In addition, in 3 cases of diffuse myocarditis and in 3 of focal involvement sections were stained for bacteria, but no organisms could be demonstrated.

Some of the clinical records contained evidence of an infectious process that was present some time prior to the onset of the final illness. This fact, together with the difficulty of demonstrating any organisms

TABLE 5.—*Leukocyte Counts*

Leukocytes per Cubic Millimeter	Cases
5,000 to 10,000	3
10,000 to 20,000	13
20,000 to 30,000	5
30,000 to 40,000	2

in the heart, suggests that the bacteria exert their influence from a distant focus by means of their toxins rather than by direct invasion of the myocardium. This mechanism of bacterial action is consistent with the occurrence of myocarditis in scarlet fever and in diphtheria.

SUMMARY

Thirty-six cases of nonspecific myocarditis were found in 3,800 consecutive autopsies. The lesions appeared to be primarily interstitial in nature and varied considerably in their cellular composition. A constant finding was the presence of elongated cells with distorted nuclei, morphologically not identical with Aschoff cells or with Anitschkow's myocytes. Four cases in which only minimal foci were observed were included since it appeared likely that in them the changes might represent early stages of more extensive myocardial involvement. Extracardiac inflammatory lesions were found in all cases except 1. No other constant findings were present that could be regarded as possible causal factors.

19. Didion, H.: Virchows Arch. f. path. Anat. **310**:85, 1943.
20. Schenken, J. R., and Heibner, W. C.: Am. Heart J. **29**:754, 1945.

Case Reports

TUMORS OF THE THYMUS

GERALD H. COORAY, COLOMBO, CEYLON

TUMORS of the thymus are of interest to clinicians and pathologists. To the former they have presented problems of diagnosis (as the case to be reported illustrates). To the latter they have presented certain difficulties with regard to their classification on an anatomic basis.

Thymic tumors may originate from the cells constituting the parenchyma, viz., the reticulum cells, the small thymic cells (the so-called thymic lymphocytes) or the corpuscles of Hassall. Primary tumors may also arise from the cells of the stroma and then may be in the nature of lymphosarcoma, fibrosarcoma, myxosarcoma or lipoma. Still another class of tumors has been described by Sternberg.[1] These apparently arise from the thymic parenchyma and are subsequently followed by a leukemic blood picture.

Wu[2] pointed out that the thymus is essentially an epithelial reticulum infiltrated by lymphocytes. He looked on the gland as a lymphoepithelial organ like the pharyngeal, faucial and laryngeal tonsils and the solitary lymph follicles of the pharynx, whose surface epithelium is normally infiltrated by lymphocytes. He described an innocent thymic tumor—diagnosed as lymphoepithelioma—with a characteristic morphologic aspect, composed of syncytial strands or cords of epithelial cells with varying numbers of lymphocytes. He also reviewed reports of 9 tumors diagnosed as lymphoepithelioma by previous writers, one tumor being cancerous, and he extracted from the literature reports of 10 thymic tumors given other designations in which the histologic description was compatible with the appearance of lymphoepithelioma. Of these 10 tumors, 2 were cancerous.

Margolis [3] expressed the opinion that there is no justification for subdividing tumors of the parenchyma into carcinoma and lymphosarcoma in the absence of conclusive knowledge of the histogenesis of all of the constituents of the thymus. He therefore referred to thymic tumors by the generic term "thymoma"—a term originally introduced by Grandhomme.[4]

The difficulties experienced by various observers in their attempts to classify thymic tumors on an anatomic basis have thus resulted in a variety of names being used for the designation of these tumors.

From the Department of Pathology, University of Ceylon.

1. Sternberg, C.: Wien. klin. Wchnschr. **21**:475, 1908.

2. Wu, T. T.: J. Path. & Bact. **61**:351, 1935.

3. Margolis, H. M.: Am. J. Cancer **15**:2106, 1931.

4. Grandhomme, F., cited by Wu,[2] p. 363.

REPORT OF A CASE

A fairly well built man aged 45 years, a laborer, was admitted to the General Hospital, Colombo, on Aug. 14, 1945 with difficulty in breathing of three days' duration. The onset was sudden. He had suffered from hoarseness for the past eight months. There was no previous history of muscular weakness. There was marked dyspnea with inspiratory stridor and cyanosis. The veins in the neck were engorged. There were no swellings in the neck. Indirect laryngoscopy showed paralysis of the vocal cords. There were no other paralyses. Low tracheotomy was performed to relieve the dyspnea, which however continued. The patient was examined fluoroscopically on the third day. A large pulsating mass was seen occupying the anterior mediastinum (fig. 1). On account of the position of the shadow and the pulsation, a diagnosis of aortic aneurysm was made. The Wassermann and Kahn tests were negative. A white blood cell count showed 9,200 leukocytes per cubic millimeter with a differential count of 68 per cent polymorphonuclears, 22 per cent lymphocytes and 10 per cent eosinophils. The dyspnea increased, and the patient died on August 21.

Necropsy.—The body was that of a fairly well nourished person of about the age stated. There was pallor of the skin and the conjunctivas. The tips of the fingers showed cyanosis. There was a recent tracheotomy wound. The pericardial cavity contained about half an ounce (15 cc.) of blood-stained fluid. In the upper and anterior mediastinum was a tumor measuring 3½ by 2½ inches (9 by 6.5 cm.). It extended from the roots of the pulmonary artery and the aorta to a point about ½ inch (1 cm.) below the tracheotomy wound (fig. 2). The tumor extended slightly over, and was adherent to, the pericardium. The ascending portion of the aorta passed through it. It was adherent to both lungs and was pressing on the right eparterial bronchus. There was a certain degree of bronchial dilatation in the upper lobe of the right lung with fibrosis (fig. 1). The tumor was encapsulated, but the penetration of the capsule by tumor tissue at several points gave rise to an irregular surface. The cut surface had a yellowish white appearance and in parts resembled fatty tissue. There were yellowish white deposits of tumor tissue over the left side of the trachea. It was noted that the main tumor mass extended in the form of a cord toward the thyroid gland. A careful examination with the naked eye, however, failed to reveal any deposits of tumor tissue in the substance of the thyroid gland.

Histologic Examination.—The type cell of the tumor resembled an epithelium or a reticulum cell (fig. 3), but the general pattern of the tumor varied in different parts on account of the variability of the cell arrangement, the presence of necrosis and degenerative changes in the cells and the arrangement of the fibrous tissue. Most of the cells were polygonal; some were ovoid or round. The nuclei were large and vesicular, with distinct nucleoli. The cytoplasm was pale pink. Multinucleated cells were frequent, and mitotic figures were seen in large numbers. In certain parts the tumor cells bore a close resemblance to squamous epithelium, but no intercellular bridges were seen.

The tumor cells were arranged in solid alveoli, the walls of which were formed of thick bands of fibrous tissue. In certain parts the cells occurred in small groups and strands, and in others large areas composed of sheets of proliferating cells were seen.

The arrangement of the tumor cells thus gave rise to different histologic patterns. Degenerative changes in the tumor cells, such as pyknosis, were evident in places. Necrosis was a marked feature. Large areas of necrosis infiltrated by polymorphonuclear leukocytes were seen in almost every section.

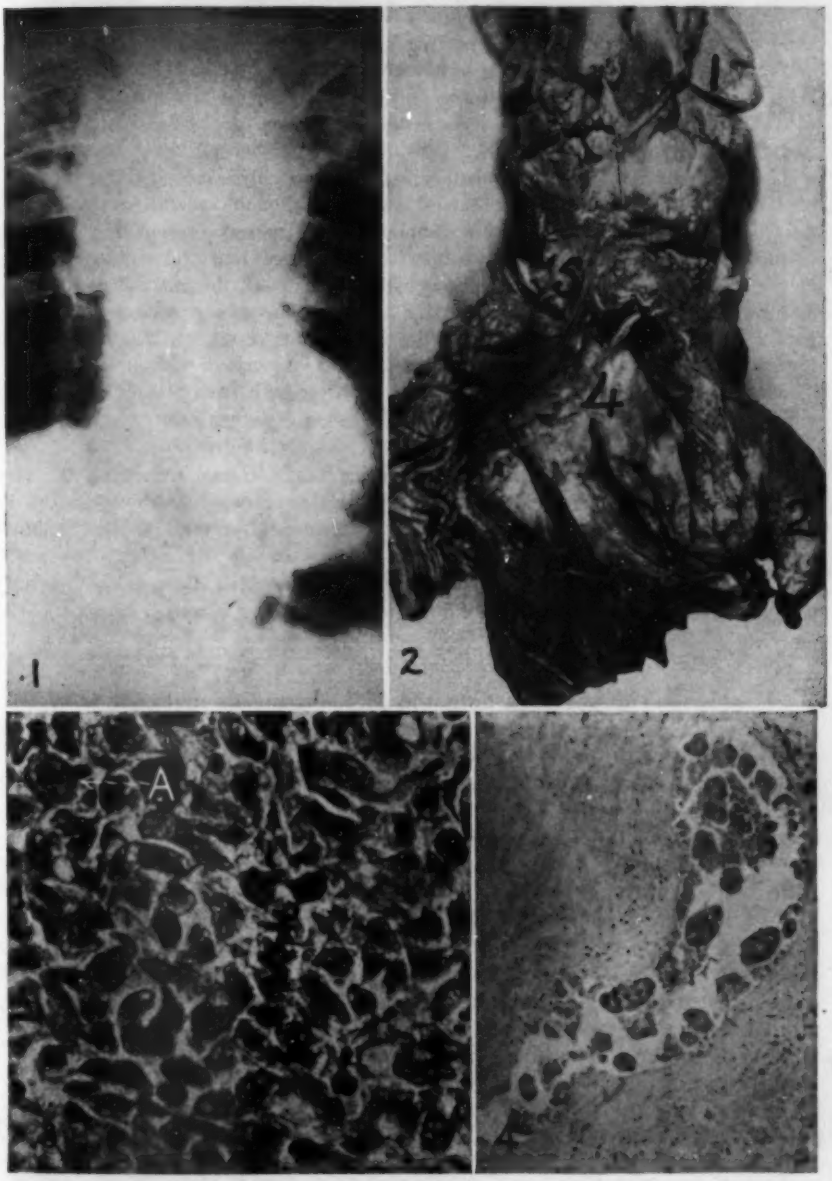


Fig. 1.—Roentgenogram showing a large mass in the anterior mediastinum. Note also the fibrosis at the right apex.

Fig. 2.—Appearance of the tumor at necropsy: (1) thyroid gland; (2) arch of the aorta; (3) pericardium; (4) tumor; (5) cordlike extension of the growth toward the thyroid gland.

Fig. 3.—Section of the tumor showing the cells of which it was composed. A mitotic figure is seen at *A*, top on the left. × 450.

Fig. 4.—Tumor cells in a blood vessel. × 100.

The stroma was composed of fibrofatty tissue, the fibrous tissue being in abundance. It was cellular in some parts and dense and hyaline in others. In certain parts the fibrous tissue formed the boundaries of alveoli containing tumor cells, and in others there was diffuse fibrosis, the fibrous tissue separating the tumor cells into small groups. Lymphocytes were few and were scattered here and there amidst the tumor cells. Only a small number of blood vessels were seen, and these, too, were rudimentary. (This probably explained the presence of large areas of necrosis and hemorrhage.) Although several sections from different parts of the tumor were examined, no structures resembling Hasall's corpuscles were seen. There was histologic evidence that the right lung and pleura, the pretracheal and tracheobronchial lymph nodes, the extrapericardial fat and the thyroid gland were infiltrated. Although the tumor encircled the first part of the aorta, it did not infiltrate the wall of the vessel. In the case of the lung and the pleura the tumor cell invasion was associated with much fibrosis. The lymph nodes were completely replaced by growth, the greater portion of which had undergone necrosis, leaving small groups of tumor cells here and there. The capsule and the periglandular tissue, too, showed infiltration.

Necrosis was a marked feature also of the tumor tissue infiltrating the fat. Here the cells were arranged in alveoli, strands or groups. Although involvement of the thyroid gland was not evident at autopsy, sections showed definite infiltration of the gland vesicles as well as of the pretracheal muscles.

In all these secondary deposits the tumor cells resembled those of the primary growth. There were innumerable mitotic figures and tumor emboli (fig. 4).

COMMENT

There is no doubt regarding the high degree of malignancy of the tumor. The large number of mitoses, the infiltration of several tissues and the presence of tumor emboli are capable of but one interpretation, that the tumor was highly cancerous.

There is no resemblance of this tumor to the lymphoepithelioma described by Wu.[2] Although the tumor cells resembled epithelium and grew in masses, the lymphocytes were few and may well have represented the lymphocytic elements of the thymic stroma.

As regards its histogenetic source, the difficulties encountered by Margolis[3] do not appear to arise in this case. According to him, tumors whose cells are morphologically indistinguishable from lymphocytes may originate either from the stromal lymphocytes of the gland or from the small thymic cells in the gland parenchyma. In this case the tumor cells were morphologically quite different from lymphocytes or small thymic cells. The histologic features which have to be considered in determining the source of the tumor are (1) the striking resemblance of the tumor cells to the reticulum cells of the thymus or to epithelium, (2) their arrangement in distinct alveoli or solid nests, (3) the paucity of lymphocytes and (4) the marked fibrosis. These appearances indicate that the neoplasm was distinctly epithelial and had started in all probability from the thymic reticulum cells, which are derived from epithelium. According to Arey,[5] the primordia of the thymus appear toward the end of the sixth week of intrauterine life as ventral saccula-

5. Arey, L. B.: Developmental Anatomy, Philadelphia, W. B. Saunders Company, 1938, pp. 190-192.

tions of the third pair of pharyngeal pouches. The sacculations are hollow at first but rapidly become solid epithelial strands. By the tenth week the orginal epithelium is transforming into a supportive framework of reticular tissue. The interpretation of the morphologic appearance of this tumor in the light of knowledge of the epithelial origin of the thymic reticulum cells leaves no doubt that its histogenetic source is the thymic reticulum.

Some noteworthy features which deserve comment are:

1. The infiltration of the thyroid gland. Although macroscopic examination failed to reveal secondary deposits, there was unmistakable microscopic evidence of infiltration of this gland. The extension of the growth in the form of a cord along the trachea toward the thyroid gland is significant. This growth may represent either involvement by the tumor of aberrant thymic nodules, which are specks of thymic tissue left behind by the gland during its descent into the thorax (Crotti [6]), or the distention by tumor cells of the "closed" system of lymphatic vessels between the thyroid and the thymus described by Williamson and Pearse,[7] the existence of which, however, has been challenged by other workers (Crotti [6]).

2. The position of the tumor—surrounding the first part of the aorta —which gave rise to the skiagraphic appearance of an aortic aneurysm.

3. The absence of (*a*) symptoms of myasthenia gravis, (*b*) any abnormality of the sexual glands.

SUMMARY

A review of the literature reveals the difficulties experienced by various authors in the classification of thymic neoplasms.

A tumor of the thymus infiltrating several tissues, including the thyroid gland, is reported. On account of its anatomic relationship to the first part of the aorta, there was an appearance of pulsation on fluoroscopic examination which was mistaken for the pulsation of an aortic aneurysm. Reasons have been adduced to show that the tumor originated from the reticulum cells of the thymus.

6. Crotti, A.: Diseases of the Thyroid, Parathyroid and Thymus, Philadelphia, Lea & Febiger, 1938.

7. Williamson, G. S., and Pearse, H. W.: Brit. J. Surg. **17**:529, 1930.

PRIMARY CARCINOMA OF THE DUODENAL BULB

S. J. RUDEN, M.D., BEVERLY HILLS, CALIF.

SUPRAPAPILLARY carcinoma of the duodenum is a rare disease. Stewart and Lieber [1] found 1 case in 3,526 autopsies and 4 more cases in 20,176 autopsies. Our case, proved to be one of carcinoma of the duodenal bulb, represents 1 case in 4,786 autopsies.

REPORT OF A CASE

A white woman aged 64 was admitted to the service of Dr. L. D. McGuire at the Creighton Memorial St. Joseph Hospital, March 8, 1946, complaining of constant pain in the right groin which radiated to the leg. There was no abdominal pain, nausea or vomiting, and there was no history of tarry stools. The appetite was good, but the patient had lost 16 pounds (7 Kg.) of weight "recently." The patient had had one previous admission about a month before and was hospitalized at that time for eleven days for thrombophlebitis involving the right lower extremity. Examination revealed a well developed but undernourished elderly white woman. Her temperature was 98 F., pulse rate 68 and blood pressure 120 systolic and 82 diastolic. There was slight tenderness in the right upper quadrant of the abdomen, but there were no palpable masses. The solid organs were not palpable. On the lateral aspect of the right arm there was a single pea-sized subcutaneous nodule, which was firm and movable. The red blood cell count was 3,750,000; the hemoglobin content 78 per cent. The white blood cell count was 10,750, with eosinophils 2 per cent, monocytes 1 per cent, lymphocytes 28 per cent and neutrophils 69 per cent. Several urinalyses gave normal results except that one specimen showed a faint trace of albumin. On March 9, 1946 a biopsy of the nodule was made and reported as showing "grade III metastatic adenocarcinoma of the skin." On March 11, 1946 the patient complained of sharp pain in the epigastrium, which lasted only a short time. This was the first time the patient had experienced such pain. Eleven days later there was a similar episode of epigastric pain, but this time it radiated posteriorly to the back. The subsequent course of the patient was rapidly downhill, with a short period of coma prior to death.

Autopsy.—Gross Examination: The body was that of a well developed but undernourished elderly white woman. There was no jaundice. The primary lesion was found in the first portion of the duodenum. In the middle of the posterior wall of the bulb there was a rounded 1 cm. area with raised hard edges. The mucosa of the center of this area was ulcerated and red and measured about 3 mm. in diameter. The remainder of the mucosa was gray-white. The liver weighed 1,550 Gm. and was studded both on the outer and the cut surfaces with a large number of firm white nodules, which varied markedly in size, the largest measuring about 3 cm. in diameter. On the lateral side of the common duct there

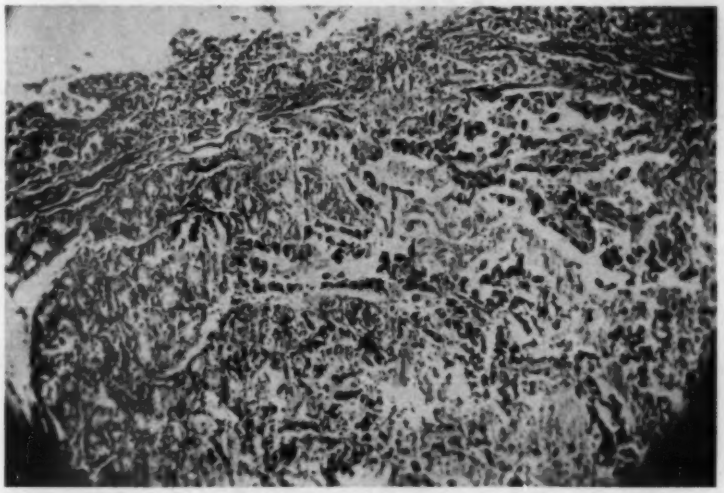
---

From the Department of Pathology and Bacteriology, Creighton University School of Medicine; Dr. B. Carl Russum, professor.

1. Stewart, H. L., and Lieber, M. M.: Arch. Surg. **35**:99, 1937.

was a single ovoid lymph node, which measured 1 by 2.5 cm. The node was firm and on cut surface a uniform pale white. The common duct was not dilated. Tumor tissue was not definitely identified grossly in any of the other organs, although microscopically tumor was found in both lungs, both kidneys and the medulla of one adrenal gland.

Microscopic Examination: Sections through the lesion of the bulb showed in the mucosa many scattered small acini lined by cancerous cells. These cells were generally large and hyperchromatic and differed considerably in shape. The nuclei varied from vesicular to pyknotic, and they were all large. Some mitotic figures were seen. There was ulceration of the mucosa with a diffuse sprinkling of polymorphonuclears throughout this layer. The submucosa showed extensive destruction of Brunner's glands and replacement with tumor, which was made up of poorly formed acini. These acini were of various sizes and shapes and generally larger than those seen in the mucosa. Small, irregular islets of tumor cells were



Duodenal adenocarcinoma.

also seen. The individual cells, however, were similar to those seen in the mucosa. The stroma was of a fine fibrous variety and interrupted by amorphous acidophilic debris and red corpuscles (fig. 1). There was no invasion of the muscularis in any of the sections studied. In other organs in which tumor was found, the histologic picture was essentially the same as in the original tumor.

The final diagnosis was adenocarcinoma of the first portion of the duodenum with metastases in a regional lymph node, the liver, both lungs, both kidneys, the medulla of one adrenal gland and the subcutaneous tissue of the right arm.

COMMENT

Stewart and Lieber [1] collected 35 acceptable cases in the literature of primary suprapapillary carcinoma of the duodenum and added 6 original cases. Berger and Koppelman [2] added 14 more acceptable cases. Since 1942 we have found 8 cases, and to this we are adding our case,

2. Berger, L., and Koppelman, H.: Ann. Surg. **116**:738, 1942.

Eight Cases of Carcinoma of the Duodenum

	Author	Age	Sex	Symptoms	Roentgenographic Findings	Palpable Mass	Occult Blood in Stool	Comment
1.	Hartzell, H. V.: Radiology **39**: 474, 1942	69	F	Anorexia, weakness and nausea	Palpable mass corresponding to duodenal bulb, which showed constant filling defect, irregular mucosal pattern and 6 hr. retention	+	+	Autopsy: Pyloric ring formed base of mass extending 6 cm. down duodenum; mass nodular and almost occlusive; extension to liver. Diagnosis: Adenocarcinoma
2.	Burke, E.; Perkel, L. L., and Gnassi, A. M.: Am. J. Surg. **62**: 267, 1943	27	M	Epigastric pain after eating, relieved by soda; "hemorrhage from duodenal ulcer." Several admissions, one for perforation	Deformity with ulcer crater	0	+	Operation: Gallbladder adherent to anterior wall of duodenum, under which was perforated ulcer with gallbladder as floor. Opposite posterior wall another ulcer had burrowed into pancreas. Patient well in 1943
3.	Ritvo, M., and Hewes, F. L.: Radiology **38**: 7, 1942	64	M	Epigastric pain relieved by milk, soda and crackers; loss of weight	Extensive irregularity of cap, "ulcerative in character"	0	?	Autopsy: 4 cm. ulcerative lesion 1st portion of duodenum: adenocarcinoma
4.	Cohn, I.: Ann. Surg. **119**: 342, 1944	71	F	Pain in right upper quadrant; intermittent vomiting unaccompanied by nausea; loss of 20 lb. (9 Kg.)	Dilatation of first portion of duodenum with altered mucosal pattern and retention of barium sulfate	+	—	Operation: 5 × 4 cm. pedunculated mass just distal to pyloric ring; enlarged nodes in greater omentum along greater curvature; microscopically, "involvement of the nodes"
5.	Cohn, I.: Ann. Surg. **119**: 342, 1944	62	M	Abdominal pain	Sharply demarcated filling defect in descending duodenum proximal to ampulla	0	?	Operation: 2 in. (5 cm.) beyond pylorus medially, an infiltrating mass, 1½ in. (4 cm.)
*6.	Laipply, T. C.: Ohio State M. J. **39**: 50, 1943	73	F	Epigastric pain, nausea and vomiting; loss 25 lb. (11 Kg.) in 6 mo.; icterus	Gastric retention; deformity and spasm of bulb, with niche of lesser curvature of bulb	+	?	Autopsy: Adenocarcinoma of suprapapillary portion of duodenum: extension to surrounding fibroadipose tissue, common duct, stomach, pancreas; metastases in liver and hepatic nodes; obstruction of biliary tree, cholangitis, multiple abscesses of liver, bile nephrosis; squamous carcinoma of cervix
7.	Bajer, I.: M. Press **214**: 46, 1945	25	M	Epigastric discomfort not affected by food; fatigue; anorexia; loss of weight, and later epigastric pain radiating to back	Residue in third portion of the duodenum	0	+	Operation: 6 cm. bleeding papillomatous growth partially obstructing lumen in 1st portion of duodenum: "cellular glandular carcinoma"
8.	Thorstad, M. J.; Gardner, L. W., and Reveno, W. S.: Harper Hosp. Bull. **2**: 12, 1944	67	F	At onset, several bouts of painless jaundice; loss of appetite, weakness and progressive loss of weight	Small diverticulum of third portion of duodenum only finding mentioned in connection with this portion of gastrointestinal tract	0	+	Autopsy: Round ulcerating 4 × 4 × 3 cm. tumor encroaching on ampulla: adenocarcinoma with colloid changes (not tumor of Brunner's glands)

making a total of approximately 64 cases of primary suprapapillary carcinoma of the duodenum reported in the literature.

Analysis of the last 8 cases reveals that: 1. The symptom complex varies from "abdominal pain" to symptoms of peptic ulcer. It is interesting to note that in cases 2 and 3 (table), in which the symptom complex simulated that of a peptic ulcer, the lesion found was essentially an ulcer. In the remainder of the cases the lesion was papillary, and the symptoms were indefinite. 2. In most of the cases there were roentgenographic findings of deformity of the duodenum, filling defect or ulcer crater, or a combination of all three. 3. Other findings, such as a palpable epigastric mass and a test showing occult blood in the stool, varied.

In our case symptoms somewhat suggestive of peptic ulcer were late. The appearance of a subcutaneous nodule which proved to be metastatic adenocarcinoma was the first indication of the presence of a cancer. The widespread metastases revealed at autopsy would indicate that the primary tumor had been present for some time, and yet clinically there were only late symptoms referable to the gastrointestinal tract.

Microscopically, tumor was seen in the mucosa and submucosa. Only an abrupt transition was noted between normal glandular structure and tumor. There was no mucus or pigment. The appearance of the individual tumor cells resembled somewhat both the cells of Brunner's glands and the columnar epithelium of the duodenal mucosa. One is therefore unable to state definitely that this tumor arose from any particular type of epithelium of the duodenum.

SUMMARY

A case of primary carcinoma of the first portion of the duodenum is presented.

In 2 of the 8 cases collected from the recent literature, there were symptoms of peptic ulcer. The lesion found in these 2 cases was essentially an ulcerative lesion. In the remainder of the cases the symptom complex was indefinite, and the lesion found was a papillary growth.

In our case there were no symptoms referable to the gastrointestinal tract until late. A single metastatic nodule in the subcutaneous tissue of an arm was the only clue to the presence of a carcinoma.

No decision could be reached as to the type of epithelial cell of the duodenum from which the tumor originated.

426 South Camden Drive.

Laboratory Methods and Technical Notes

A SIMPLIFICATION OF THE TECHNIC FOR DEMONSTRATING ALKALINE AND ACID PHOSPHATASE IN TISSUES

J. D. BARGER, M.D., ROCHESTER, MINN.
Fellow in Pathology, Mayo Foundation

THE HISTOCHEMICAL methods demonstrating alkaline phosphatase, first described by Gomori [1] and by Takamatsu,[2] and acid phosphatase (Gomori [3]) have become, with certain minor modifications, routine to the experimental pathologist and cytologist. The existing technics, while relatively simple, require that the buffered staining solutions be prepared from a series of previously prepared stock solutions. Those for the alkaline phosphatase method are quite different from those for the acid phosphatase.

The method described in this paper is a simplification of the existing technics in that the stock buffer solution for each enzyme is made up with the metallic ion which acts as the indicator, the activator of the enzyme and the buffer all in one solution. The substrate is so prepared that it may be used with either the buffered stock solution for alkaline or that for acid phosphatase.

TECHNIC

I. SOLUTIONS

A. Alkaline phosphatase buffer stock solution

Barbital sodium	5.0 Gm.
Calcium chloride	5.0 Gm.
Magnesium chloride	0.5 Gm.
Water, distilled	1,000.0 cc.

It is advisable to check the p_H and adjust to p_H 9.4 if necessary.

B. Acid phosphatase buffer stock solution

Sodium acetate	27.2 Gm.
Acetic acid, glacial	12.0 cc.
Lead nitrate	5.0 Gm.
Water, distilled	1,000.0 cc.

It is advisable to check the p_H and adjust to 4.5 to 5.0 if necessary.

C. Stock glycerophosphate substrate solution

Sodium glycerophosphate (52 per cent alpha)	20.0 Gm.
Water, distilled	1,000.0 cc.

About 5 drops of chloroform are added to each of the foregoing solutions as a preservative, and the solutions are stored in the refrigerator when not in use.

1. Gomori, G.: Proc. Soc. Exper. Biol. & Med. **42**:23, 1939.
2. Takamatsu, H.: Tr. Soc. path. jap. **29**:492, 1939.
3. Gomori, G.: Arch. Path. **32**:189, 1941.

For use, the following buffer–substrate mixtures are prepared from the foregoing stock solutions.

D. Alkaline phosphatase buffer–substrate mixture

Alkaline phosphatase buffer stock solution (solution A).... 4 parts
Stock glycerophosphate substrate solution (solution C).... 1 part

E. Acid phosphatase buffer–substrate mixture

Acid phosphatase buffer stock solution (solution B).....10 parts
Stock glycerophosphate substrate solution (solution C).... 3 parts

II. STAINING

A. Alkaline phosphatase

1. Fix sections of tissue cut 2 to 3 mm. thick in cold acetone (4 C. or less) for twenty-four hours and then impregnate at room temperature with 5 per cent solution of cellulose acetate in acetone for six hours.

2. Drain the excess cellulose acetate from the pieces of tissue and transfer tissue to xylene for one hour.

3. Embed in paraffin at 54 to 56 C. for sixty minutes and prepare paraffin blocks in the usual manner.

4. Cut sections at 5 to 8 microns and mount on slides with albumin-glycerin. The tissue is fixed to the slide by placing in an incubator at 37 C. for five to eighteen hours.

5. Remove paraffin from sections by the usual method with xylene, absolute alcohol or acetone and 95 per cent alcohol.

6. Incubate the sections in the alkaline phosphatase buffer–substrate mixture (solution D) at 37 C. for one to twenty-four hours, depending on the concentration of enzyme in the tissue.

A duplicate set of sections to serve as controls is incubated under the same conditions for the same length of time in the alkaline phosphatase buffer stock solution with distilled water substituted for the stock glycerophosphate substrate solution.

7. Rinse rapidly in distilled water.

8. Immerse sections in 5 per cent aqueous solution of silver nitrate and expose to light. Sunlight may be used. This requires up to one hour's exposure. The use of a General Electric "mazda RS sunlamp," 275 watts, as a source of ultraviolet radiation is ideal. With the latter method, two minutes' exposure is recommended.

9. Fix reduced silver by placing the sections in a 5 per cent aqueous solution of sodium thiosulfate for one minute and wash under running tap water for five minutes or in about ten changes of water.

10. Counterstain lightly, if desired, with either Harris' hematoxylin or 1 per cent aqueous light green SF. If hematoxylin is used, wash in water after staining, and if light green is used, differentiate quickly in alcohol.

11. Dehydrate in 95 per cent alcohol, followed by absolute alcohol or acetone.

12. Clear in xylene and mount in balsam or "clarite."

Results.—The sites of alkaline phosphatase activity stain golden or dark brown to black.

B. Acid phosphatase

Steps 1 through 5 are identical with those for alkaline phosphatase described previously.

6. Incubate the sections in the acid phosphatase buffer–substrate mixture (solution E) at 37 C. for six to twenty-four hours, depending on the enzyme concentration in the tissue.

A duplicate set of sections to serve as controls is incubated under the same conditions for the same length of time in acid phosphatase buffer stock solution with distilled water substituted for the stock glycerophosphate substrate solution.

7. Rinse in distilled water.

8. Rinse in 2 per cent acetic acid.

9. Wash thoroughly in distilled water.

10. Immerse for two minutes in a dilute solution of ammonium sulfide and wash under tap for five minutes.

11. Counterstain as, and if, desired (see step 10 under method for alkaline phosphatase), dehydrate and mount in balsam or "clarite."

Results.—The sites of acid phosphatase activity stain dark brown to black.

COMMENT

The histochemical methods described are used routinely in the laboratory of the section on pathologic anatomy of the Mayo Clinic to demonstrate alkaline or acid phosphatase activity or both. More than 1,000 sections have been successfully stained by these procedures. With human tissues, good results have been obtained even as long as twenty-four hours post mortem and even though the tissues were taken from bodies in which there had been arterial embalming prior to necropsy.

It has been found that the combining of the buffers with the calcium and lead salts, for alkaline and acid phosphatase, respectively, reduces the number of reagents needed in the immediate preparation for the staining, simplifies the procedure, reduces the possibility of error and gives uniformly good results. The glycerophosphate solution is reasonably stable when stored in the refrigerator, and the buffer-substrate mixtures are always clear.

In the final steps of the demonstration of alkaline phosphatase, we at the Mayo Clinic employ in essence the von Kossa[4] method for calcium. As a source of light, we use a General Electric "mazda RS sunlamp" 275 watts) instead of daylight, a procedure which principally produces uniformity of exposure and is of time-saving value, simultaneously. The lamp is mounted in a wooden box (figure) constructed for this purpose. The staining dish containing the slide is introduced beneath the light on the tray at the bottom of the box. The box is of simple construction, and the materials used are easily obtainable. There are no rigid specifications; it can be designed to meet the needs of the individual laboratory. The one we use measures 15 by 9½ by 9 inches (38 by 24 by 23 cm.), in height, depth and width, respectively.

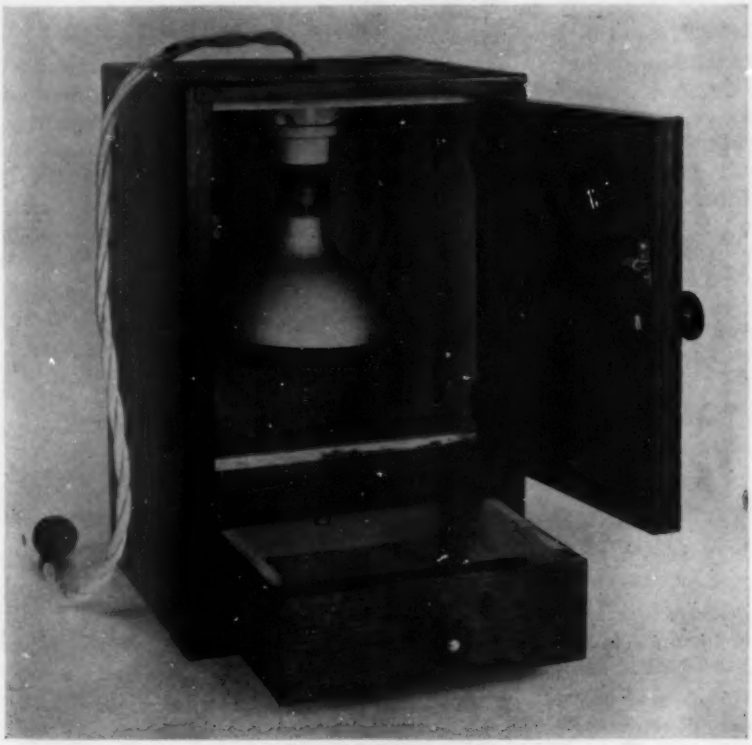
The sites of alkaline phosphatase activity are readily demonstrated. The results of the procedure for demonstrating acid phosphatase are less uniform and at times disappointing unless meticulous attention to detail is observed. In working with this enzyme, one should empha-

4. von Kossa, J.: Beitr. z. path. Anat. u. z. allg. Path. **29**:163, 1901

size particularly fixing of the tissues at low temperatures and not departing from the staining technic given. Moog [5] has shown that ascorbic acid in one hundreth–molar concentration will activate the acid phosphatase, and this may be added to the acid phosphatase buffer stock solution, particularly if it is anticipated that the concentration of the enzyme will be low.

SUMMARY

A simplification of existing technics for demonstrating alkaline and acid phosphatase is presented. The essential features of the modifi-



Front view of staining box described in the text for the von Kossa stain. The drawer for the staining dish is partially open; a door (open in the photograph) is convenient for changing the "sunlamp," when and if necessary, and for cleaning the box.

cation are such that the number of stock solutions is reduced. The solutions used in this method are stable and may be made up in large quantities; thus the necessity of preparing staining solutions from a relatively large number of stock solutions each time the test is performed is avoided. The stock glycerophosphate substrate solution is used in both the method for alkaline and that for acid phosphatase.

5. Moog, F.: Biol. Bull. **86**:51, 1944.

PROCEDURE FOR DEMONSTRATING LEPRA BACILLI IN PARAFFIN SECTIONS

G. L. FITE, M.D., P. J. CAMBRE and M. H. TURNER, B.S., CARVILLE, LA.

THE lesser degree of acid-fastness of lepra bacilli as compared with tubercle bacilli appears in a disagreeable manner in the difficulty of demonstrating the organisms of leprosy in paraffinized tissues.[1] Faraco[2] showed that by ordinary methods of demonstrating acid-fast organisms the lepra bacilli are often not acid fast, are not differentiated and may be stained by the counterstain. He devised a method of oiling the sections and staining with carbolfuchsin while the sections contained oil. Under these conditions the bacilli retained this dye. The method is effective but awkward and cumbersome. In working with similar procedures it has been found that staining before removal of the paraffin is satisfactory (though impracticable) and that replacement of the paraffin with a light oil produces still better results. The following procedure, which is not exactly a new method, has proved most valuable in demonstrating lepra bacilli in tissues, irrespective of the technics of fixation and embedding. It succeeds admirably with tissues indifferently fixed or embedded years previously where other procedures fail miserably.

PROCEDURE

1. Remove paraffin with two changes of the following mixture, allowing a minute or two for each change:

Cottonseed (or peanut or olive) oil	1 part
Xylene	2 parts

2. Drain, wipe off excess oil and blot to opacity. The residual oil in the section helps to prevent shrinkage and injury of the section.

3. Remove mercury crystals (if present) with strong solution of iodine, U. S. P. (two minutes), followed by a hyposulfite or thiosulphate solution rather than alcohol. Wash in tap water.

4. Stain cold fifteen to thirty minutes in any standard preparation of carbolfuchsin (Ziehl-Neelsen) but not in a concentrated solution such as Kinyoun's. Wash.

5. Decolorize with 1 per cent concentrated hydrochloric acid added to 70 per cent alcohol—not to the point of totality, but leaving a faint pink color. One to two minutes will be required. Wash.

6. Counterstain with Loeffler's alkaline methylene blue about thirty seconds. Wash in tap water.

From the Pathology Laboratory, United States Marine Hospital (The National Leprosarium), United States Public Health Service.

1. Fite, G. L.: Am. J. Path. **14**:491, 1938.

2. Faraco, J.: Rev. brasil. leprol. **6**:177, 1938.

7. Blot, let stand a few minutes to dry out well, mount directly in a synthetic mounting medium, such as "clarite" or "permount."

Almost any oil will serve the purpose, from liquid petrolatum to camphorated oil, although the volatile oils are less useful. The oil slows up the various steps a little, but not importantly. It hastens the acid-fast staining, however, so that the use of heat is preferably avoided, more even regular staining resulting without it. A larger proportion of oil in the xylene makes even decolorization difficult. It is possible to take sections to water by routine methods and then oil them, as Faraco did, but it is difficult to obtain even staining thereafter. The procedure as given is equally effective with the bacilli of rat leprosy and should do well with tubercle bacilli. It is particularly recommended for tissues containing the organisms of human leprosy.

Notes and News

Appointments, Etc.—Stanhope Bayne-Jones has resigned as professor of bacteriology in Yale University and as director of the board of scientific advisers of the Jane Coffin Childs Memorial Fund for Medical Research to accept the position of president of the joint administrative board of the New York Hospital-Cornell University Medical Center. His address now is 525 East Sixty-Eighth Street, New York.

Laurence H. Snyder, professor of zoology and of medical genetics at Ohio State University, has accepted the deanship of the graduate school of the University of Oklahoma and the professorship of genetics of the college of medicine.

In the United States Public Health Service Leonard Scheele has been appointed chief of the National Cancer Institute in succession to R. R. Spencer, who resigned to devote himself to biologic research and to the training of young physicians in the enlarged cancer program of the institute.

Central Laboratory.—The Veterans Administration has established a central pathologic laboratory in Washington, D. C., in cooperation with the Army Institute of Pathology, to provide more facilities for consultation, training and research in pathology.

Research Fellowships.—The National Foundation for Infantile Paralysis, Inc., has made a grant in support of research fellowships in fields related to infantile paralysis. Fellowships are offered to provide (1) opportunities for training and research in those basic medical sciences which will be of particular value in furthering progress in the field of orthopedic surgery and (2) special experience in the study of virus diseases. Senior fellowships in the fields of orthopedic surgery, pediatrics and virus diseases, open to men and women who have already shown definite achievement in research, are also offered under this grant. The chairman of the board in charge of these fellowships is Robert F. Loeb, Columbia University, New York.

The American Institute of Biological Sciences.—This institute is to be set up within the framework of the Division of Biology and Agriculture of the National Research Council, which is financing the preliminary organization. H. B. Steinbach, Washington University, St. Louis, with headquarters at the National Research Council, is acting as executive secretary during the period of organization.

New Journal.—A new periodical on pathology and clinical medicine is published by the department of pathology, Hospital 2 de Mayo, Lima, Peru, under the title *Archivos peruanos de patologia y clinica*. The editor is Dr. Oscar Urteaga-Ballon.

The American Society for the Study of Arteriosclerosis.—This society has been organized with a membership of about 80 and with W. C. Hueper, 113 West Eighteenth Street, New York, as president, William B. Kountz, St. Louis, as vice president, and O. J. Pollak, Wilmington, Del., as secretary-treasurer. The directors are: E. Cowles Andrus, Baltimore; G. Lyman Duff, Montreal, Canada; Harry Goldblatt, Los Angeles; George R. Herrmann, Galveston, Texas; Louis N. Katz, Chicago; Irvine H. Page, Cleveland. It is planned to hold a meeting in Chicago this fall.

Books Received

HIPPOCRATIC WISDOM: FOR HIM WHO WISHES TO PURSUE PROPERLY THE SCIENCE OF MEDICINE. A MODERN APPRECIATION OF ANCIENT SCIENTIFIC ACHIEVEMENT. By William F. Petersen, M.D. Pp. 263, illustrated. Price $5. Springfield, Ill.: Charles C Thomas, Publisher, 1946.

"Hippocratic Wisdom" is a modern appreciation of ancient scientific achievement. The book voices and advocates very winningly, and yet emphatically, the indispensable tradition and continuity of classical medical theory and practice. It analyzes the mature wisdom connected with the name of Hippocrates and shows that the approach to scientific medical knowledge was the same in classical days as it ideally is now.

Dr. Petersen is aware that all great medical achievement has grown out of a recognition of the laws of nature and of nature itself. The Greeks did not know it all, but they were naturalists in their knowledge and experience, as they turned their skill to medical problems and the art of healing. Not technicians in our modern sense, they conceived man and his functions and ailments, and the regulation or cure of these, as deeply influenced by forces and conditions in nature. That was their basic clinical view, and out of that view their observations were recorded thoughtfully, their interpretations determined and formulated.

The hippocratic texts need no more elaborate commentary than their repeated study has deposited in literature thus far. Therefore Dr. Petersen proposes in his exposition of the Wisdom no new commentarial apparatus, but emphasizes the validity of the Greek tradition as a principle of principles for those who aim to serve mankind through medicine, not merely to practice a craft of skill.

Aptly the author compares modern medical teaching to the teaching of anthropology without ecology—or, one might say, botany without plant relationship or physiology. Skill is a great virtue, as is a perfect technic, but it must not be isolated from clinical consideration and experience. Not laboratory experience alone, nor study in the library, but persistent, intelligent observation in the sick room, backed by similar experience with normal manifestations and phenomena. "In the beginning are the cases," as Henderson says; at the end should be the descriptive exposition of the case, a theory based on a wider range of uniformity, and the conceptual scheme of aiding nature's healing powers. Hippocrates knew that air is a basic requirement of tissue function and that a disturbance of this factor influences pathologic conditions and may call forth many other factors (weather, etc.) disturbing the balance of the body humors; he also had a view of compensatory reactions between the body tissues. His theory of inflammation recognizes many kinds of trauma. More interesting even than these enlightening conclusions is that with the old master came a beginning recognition of the complexity of causes of disease which deserves special attention in view of modern theories of specific causes and specific cures, but these later theories frequently prove too simple as applied to complicated processes and conditions.

So Dr. Petersen leads us through the details of the Wisdom by a spirited analysis, amplified by modern experience, of what Hippocrates specifically said and meant.

It is advisable to follow this analysis with some deliberation, for the hippocratic texts alone contain plenty of food for serious reflection and comparison, and nobody need think the author is giving us some easily digested medical-historical entertainment. The texts read easily enough in translation but appeal directly to philosophic thought, which would better not be suppressed if the intended enlightenment is to result. Nor are the author's many incisive and elucidating remarks, which bring out both contradistinctions and similitudes between medical and

philosophic thought and practice in the fifth (B. C.) versus the twentieth century, to be neglected by the reader, whether student or master of the science of medicine.

The specific analysis of the texts includes twelve chapters comprising the ideas of Hippocrates on erysipelas (as related to the seasons), anoxia, reproduction, epilepsy, hydrophobia, pneumonia and allied diseases, phthisis, the case of Silenus and other observations; surgical technic, bandaging; regional surgery, fractures and dislocations; medical theory, the principle of disease, general trauma. These chapters are followed by a resumption or corollary that brings out what may be called in old fashioned language a moral—apart from the light it throws on the hippocratic group of medical writings. We quote for once the following:

"It is possible that we, who have lived during the epoch of great therapeutic advance as well as the students who enter the field of medicine when this development is proceeding at an accelerated pace, may feel that Hippocratic wisdom is wholly out of date.

"These very advances in therapeutic intervention have led away from a broad concept of the causation of disease. Who need care about the why of disease when the remedy is immediately at hand?

"The student sees a coryza in the department of otolaryngology; a spontaneous abortion in obstetrics; an endocarditis in medicine; a retinal thrombosis in ophthalmology; a prostatic episode in the genitourinary department; a hypomania in psychiatry; a diverticulitis in surgery, or a Bell's palsy in neurology without ever being faintly conscious of the connecting link that might make an intelligible and coherent picture of the tangled skeins. He never considers the patient as a whole in the environment as a whole."

In these analyses there are interpretations which might be considered critically, and other formal details probably debatable. But on the whole, the Hippocratic *direction* does not depend on syllables and other philologic niceties; it consists in movement rather than in form.

The last hundred pages contain notes and references, a vocabulary and a well prepared table of contents.

In a way this is an astonishing book, coming, as it does, from a medical man deeply concerned in the realities of his day and yet looking back twenty-three hundred years for historic anchors by which to keep secure the ship which he shares with many other capable men. But the Greeks never fail to inspire mankind historically. As Dr. Petersen says: "Our age is one very much like the Hippocratic.—Witness the clashing impact of empires and the march of the legions —the transition state of social forms—the ambiguous shibboleths, the confusion of the patriot, and the cunning of the tyrants."

This is true, although medical men never have gone to the radical extremities practiced by politicians. Our forces of social order cannot ever be stifled. Nor should the author of this constructively provocative book be denied the opportunity he seeks through this work of inspiring his colleagues, young and old, with the dignity, the benefit, the necessity, of giving proper respects to the ideals which the muse of history preserved for our welfare.

CHARLES-ÉDOUARD BROWN-SÉQUARD: A NINETEENTH CENTURY NEUROLOGIST AND ENDOCRINOLOGIST. By J. M. D. Olmsted, M.A. (Oxon.), Ph.D., D.Sc., professor of physiology, University of California. Pp. 253. Price $3. Baltimore: The Johns Hopkins Press, 1946.

This volume contains the eighth course of lectures under the Hideyo Noguchi Lectureship of the Institute of the History of Medicine of the Johns Hopkins University. The lectureship was endowed by the late Emanuel Libman, of New York. There are three lectures, the headings of which give a good idea not only of their scopes but also of the main epochs in the life of Brown-Séquard: Mauritian student and free lance investigator in Paris (1817-1894); his neurologic practice and American professorships (1854-1878); his occupancy of the chair of medicine at the Collège de France (1878-1894). Brown-Séquard was born in Mauritius,

then under the British flag. His father, Charles Edward Brown, captain in the American Merchant Marine, was lost at sea before the son was born, and the French mother, Charlotte Séquard, was thrown on her own resources. The further story is crowded with exciting events: the study of medicine in Paris; remarkable physiologic experiments; lectures in Philadelphia, the father's birthplace, New York and Boston; brief service as professor in the Medical College of Virginia; delegate to the 1855 meeting of the American Medical Association. This visit to the United States was the first of many up to 1878 when "he had finally at the age of sixty-one come home to France" for good as the successor of Claude Bernard at the Collège de France. In concluding his scholarly biography and his circumstantial and critical analysis of Brown-Séquard's investigative work, Olmsted places him as "the last in the line of a great tradition of French experimental physiology at the Collège de France. . . . Magendie, Bernard and Brown-Séquard spanned the XIX century from its first decade to the last, and from their work came the ideas whose fulfillment now occupies much of our present day investigation." Brown-Séquard "left an indelible mark on physiology and medicine, and we look back on him as a brilliant and indefatigable investigator. . . ." Olmsted's book is welcomed as an important and timely contribution to medical biography and history.

HENRICI'S MOLDS, YEASTS AND ACTINOMYCETES: A HANDBOOK FOR STUDENTS OF BACTERIOLOGY. By Charles E. Skinner, Ph.D., assistant professor of bacteriology, University of Minnesota, Minneapolis; Chester W. Emmons, Ph.D., principal mycologist, Division of Infectious Diseases, National Institute of Health, Bethesda, Md., and Henry M. Tsuchiya, Ph.D., research associate, Division of Microbiology, Hormel Institute, University of Minnesota, Austin. Second edition. Price $5. Pp. 409, with 136 illustrations. New York: John Wiley & Sons, Inc., 1947.

Henrici's book was published first in 1930. It was received with favor. The revision begun by Henrici has been completed in thorough and competent fashion. A new chapter deals with penicillin and other antibiotic substances, and another with variations in the lower fungi. The book is well printed and illustrated. It represents fully the present state of scientific, medical and industrial mycology, and will continue to be a standard work in its field.

THE PRESERVATION OF PROTEINS BY DRYING WITH SPECIAL REFERENCE TO THE PRODUCTION OF DRIED HUMAN SERUM AND PLASMA FOR TRANSFUSION. Medical Research Council Special Report Series no. 258. By R. I. N. Greaves. Paper. Price 2 shillings. Pp. 54, with 5 figures and 20 plates. London: His Majesty's Stationery Office (New York: Library of British Information, 30 Rockefeller Plaza), 1946.

Because of experience gained in freeze-drying antiserums on a laboratory scale in the department of pathology of Cambridge University, the Medical Research Council commissioned the author in 1939 to develop efficient methods for freeze-drying the large amounts of plasma and serum likely to be needed during the war. An original pilot plant and then a final pilot plant which embodied the principles worked out by Greaves and his colleagues were produced. Following this a plant was constructed at Cambridge designed for an output of 2,500 bottles of dried material per week but which could be extended to 5,000 bottles per week. In this plant was produced most of the dried serum and plasma used by the British military forces and civilians, consisting of, up to September 1945, 318,700 bottles, each representing 400 cc. of material. An additional 32,617 bottles were produced in the pilot plants. This does not include dried diagnostic and therapeutic serums or serums and plasmas for experimental purposes.

In this monograph are set forth the theoretic considerations involved in desiccation from the frozen state and the physical principles of the processes. It also presents the design and technical details of the large drying plant. The problems of drying from the frozen state were solved by methods which in some respects differ from those used in the United States.

The primary drying in the large plant was carried out in eight large steel cylindric desiccators measuring 3 feet (91 cm.) in diameter and 6 feet (183 cm.) high. Steel plates welded to the bottom and sealed to the top with "Apiezon Q" made the chambers air tight. Suspended from the top cover is a heater head constructed to carry 180 standard M.R.C. bottles, each one in a cell surrounded by an electric coil that supplies 7 watts of current regulated to heat the bottles to + 30 C. In the bottom of the chamber is a condenser consisting of a coil of copper tubing through which is pumped brine at — 40 C. from a central ammonia refrigeration plant. Vacuum requirements are provided by two large single stage pumps connected by a manifold to the eight desiccators.

A novel way of preliminary freezing of the material to be dried is used. The bottles are rotated at a comparatively high speed on their vertical axes in giant centrifuges. In this way a cone is forced down through the liquid, which is frozen in this position by a current of air at — 18 c. The same effect is obtained as in shell freezing with liquid refrigerants. When transferred to the drying chambers and the pressure reduced rapidly enough, the liquid remains frozen until dried.

In order to produce 5,000 bottles of dried material a week the whole drying cycle, including defrosting of the condenser, is completed and restarted in forty-eight hours. At half this rate, seventy-two hours could be allowed for the drying and twenty-four hours for defrosting and servicing.

When removed from the primary desiccators, the dried protein contains 0.4 per cent residual moisture. To remove this, the bottles are transferred to smaller chambers, where they are kept four days *in vacuo* over phosphorus pentoxide. At the end of this period the vacuum is replaced by dry oxygen-free nitrogen to a slight positive pressure.

The physical factors involved in drying are automatically controlled and mechanically recorded, including the temperature developed in the bottles as measured by thermocouples, the temperature of the circulating refrigerant as measured by a platinum resistance thermometer, and the pressures produced in the chambers as measured by Pirani gages. An alarm system gives warning of a failure of electric supply, a rise of temperature of the brine or a rise of pressure in the vacuum system.

The container for the material being dried is kept bacteriologically closed throughout the whole process. No antiseptic is added. During drying in the primary chambers the mouth of the bottle is covered with a pad of cotton between two layers of gauze. The pad is replaced with a rubber diaphragm held in place with an aluminum cap. For the final drying and introduction of nitrogen a hole is punched in the metal cap and a hypodermic needle to which is attached a bacterial filter is pushed through the rubber diaphragm. The final seal is made with plasticine and by dipping the neck of the bottle in a capping solution.

The material in the monograph is presented clearly if not in an orderly manner, with excellent photographs, several charts and curves, and a good bibliography. It is indicated that further experiments are in progress which will meet certain problems, especially those introduced in the drying of sodium penicillin. A method of vacuum spin–freezing is being developed which will permit the loading of the drying apparatus with liquid material, thus rendering unnecessary all prefreezing and low temperature storage.

TUMORES Y SEUDOTUMORES DE LA MAMA. ESTUDIO DE INVESTIGACIÓN EXPERIMENTAL SU PROFILAXIS Y TRATAMIENTO. By Dr. Jacinto Moreno. Pp. 142, with 40 illustrations. Buenos Aires: López & Etchegoyen, 1946.

ANATOMÍA PATOLÓGICA DE LA INFLAMACIONES ESPECÍFICAS. Por el Doctor Ramiro Pico Duni, docente libre de la cátedra de anatomia y fisiologia patológicas de la Facultad de Ciencias Médicas de Buenos Aires, Jefe del laboratoria de anatomia patológica del Hospital Argerich. Pp. 189, with 62 illustrations. Buenos Aires: López & Etchegoyen, 1946.

INDEX TO VOLUME 43

Subject entries are made for all articles. Author entries are made for original articles. Book Reviews and Obituaries are indexed under these headings in alphabetical order under the letters B and O, respectively.